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1908-09

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PREFACE

The contents of the fourth volume of Harvey Society Lectures furnish a striking example of the many points of contact between medicine and the collateral sciences, including as they do addresses on heredity, immunity, chemistry, and osmosis.

The Editor wishes to express, as in previous volumes, his obligations for permission to reprint those lectures which have already been published in the medical journals: To the Medical Record for permission to publish Dr. Calmette's lecture; to the Archives of Internal Medicine for permission to republish the lectures of Dr. MacCallum, Dr. Lusk, Dr. Falta, Dr. Anderson and Dr. Rosenau, and Dr. Hiss; and to the Lancet for permission to republish Dr. Leathes's lecture. The latter part of Dr. Macallum's lecture appeared in the Transactions of the Royal Society of Canada. The lecture by Dr. Davenport has not previously appeared in print.



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INTESTINAL INFECTION AND IMMUNITY IN TUBERCULOSIS*

PROF. A. CALMETTE
Director of the Pasteur Institute of Lille, France

PERMIT me to express, first of all, my gratitude for the great honor which you have bestowed upon me in inviting me to discuss before your illustrious society the present state of our knowledge of the rôle of the digestive tract in tuberculous infection and in immunization against tuberculosis.

For several years this question has been the principal object of my laboratory researches, which I have pursued with the aid of some of my scholars, particularly of C. Guérin. It has engaged the attention in an equal degree of a large number of workers in every country. There are but few of greater interest, as the discussions of our recent congress in Washington will bear witness.

I do not pretend that I can cast sufficient light upon this question to warrant us in expecting in the very near future the establishment of a method capable of protecting humanity against the most terrible of the scourges which desolate it. I do wish, however, to make an effort to acquaint you with the principal experimental facts upon which we can build to-day so as better to comprehend the mechanism of the tuberculous infection, and I trust that you may be brought to regard the hopes which have been raised so many times in vain, as at least partly realizable.

It is forty-three years since Villemin announced that tuberculosis is inoculable and contagious, and twenty-six years separate us from the memorable epoch at which Robert Koch, discovering and cultivating his bacillus, made the demonstration

* Lecture delivered October 24, 1908.

of its specificity. Notwithstanding the enormous accumulation of publications in the last quarter of the century, we are still but very imperfectly informed upon the respective importance of the different ways by which the virus of tuberculosis can penetrate into the bodies of man and of susceptible animals. Until lately, the greater number of scientists, clinicians or experimental workers, have considered as an indisputable dogma the respiratory origin of pulmonary tuberculosis, and the celebrated debates which took place at the International Conferences at The Hague in 1906 and at Vienna in 1907, and since then in various learned societies, are so fresh in your minds that it would be useless to recall them. It appears, however, that the impression that a final decision had been reached is not correct, and that, as is always the case, the truth lies between two extremes.

If it appears undeniable that in certain probably very rare cases, direct infection of the lung takes place through the respired air, notwithstanding the many and very efficacious natural defenses which line this route, it is also manifest that the path to the pulmonary parenchyma normally followed by the tubercle bacillus is most often through the lymph- or blood-vessels, the great portal of entry being the digestive tract.

Experimentation has shown that to effect primary tuberculous infection of the lung by the air, it is necessary to place the animal under pathological conditions, as has been done by Nocard, Flügge, and, more recently, Kuss, in immobilizing the animals so as to oblige them to breathe for a long time an atmosphere charged with infectious particles, liquid or dry. Very fine spray is certainly dangerous, above all for young infants, and it is evident that pneumonia with early caseation and acute pulmonary tuberculosis of infants are almost always of respiratory origin. When a mother or a nurse, the subject of an active pulmonary tuberculosis, coughs or sneezes at a short distance from the lips of an infant breathing through its open mouth or crying in expectation of the breast, contagion by inhalation is all but fatal. But this mode of infection is certainly exceptional for the older child or for the adult.

Infection by dust laden with bacilli is more exceptional still; and the positive results of Cornet, as well as those of Kuss, accomplished by shutting up the immobilized animals in an inclosed space, in an atmosphere of talcum powder or of the débris of vegetable fibres mixed with dried sputum, invalidate in no way the much more numerous negative results described by Baumgarten, Tappeiner, Cadéac and Mallet, and Petersson, and by myself with Vansteenberghe.

Does not, however, the normal asepsis of the respiratory tract attest strongly the protective efficiency of the defensive apparatus of this region, when not affected by pre-existing lesions of the nasopharynx, of the larynx, and of the larger bronchi?

The primary lesions of the tracheal and bronchial nodes, which certain authors consider as always related to a respiratory infection, can no longer be invoked as a convincing argument in favor of this view. If it be true that, following the law of Parrot, the nodular lesion should always be accompanied or preceded by one or several pulmonary tubercles, there is nothing to prove that these last must be of aërial origin, since they make their appearance with great frequency in animals artificially infected through the digestive tube. Breton and myself have observed this regularly upon a great number of guinea-pigs which had absorbed through the rectum or been given by an œsophageal sound, a small quantity of a fine emulsion of a pure culture of the bacillus of bovine tuberculosis. These animals when killed after four or five weeks very often presented no other lesions than one or two superficial tubercles, as large as the head of a pin, in one of the anterior lobes of the lung, with a co-existing extensive tuberculosis of the peribronchial lymph-nodes. An observer who was ignorant of the conditions under which the infection had been experimentally produced, would undoubtedly have stated that the lesion was due to respiratory infection.

It can then hardly be denied that if primary tuberculosis of the lung or of the tracheobronchial lymph-nodes by the direct

inhalation of particles carrying bacilli is quite possible in some cases, it is certainly exceptional.

Quite frequent, on the other hand, but also less serious, is infection of the body through the digestive tract.

When I state that tuberculosis is most often contracted through the intestine, I do not wish to be considered as saying that I attribute to the food, and consequently to the milk of tuberculous cows, the essential rôle in the tuberculous infection of man. I am convinced, on the contrary, that the importance of infection through milk has been very largely exaggerated in the last few years. The fact that tuberculosis is so common, both in infants and in adults, in countries where the use cows' milk as human food is exceptional, for example, in Egypt, India, Indochina, and Japan, shows that the propagation of tuberculosis from man to man is infinitely more common than the infection of man by cattle.

Without doubt the ingestion of the milk of tuberculous cows is dangerous, principally for young children, though even for healthy adults, and much more for those in whom the intestinal mucosa is not intact, when the ingestion of virulent material is frequently repeated. But infinitely greater and more frequent is the danger from human bacilli freshly expelled from the lung of a consumptive, when these bacilli are carried by the sputum to the food or upon the mucous membrane of the mouth by direct or indirect contact of the lips, the hands, soiled objects, etc., or by flies. The tuberculous mother who tastes the dish prepared for her child or who wipes its face with her handkerchief, and the nurse who moistens her breast with her saliva, subject the infant in their care to the danger of tuberculous infection. The child who handles its food after crawling about the floor, and the man who wets his fingers to turn the pages of a book, or to handle type, or who works with tools belonging to a consumptive whose sputum contains the bacilli, may ingest at any moment a number of virulent bacteria; and the risks of contagion are much more serious the more abundant and frequently repeated these ingestions are.

And how can one doubt that the rôle of flies is particularly

important and dreadful in the unhealthy tenements where, among the sick persons, children and adults, living side by side in the closest contact, these insects swarm in compact masses alternately upon the sputum and upon the food?

Careful study of the tuberculous infection in animals furnishes abundant clinical proof of the predominance of infection through the digestive tract.

For example, it is known that the flesh-eating animals such as the lion, the tiger, the hyena, and the jackal, when confined in a menagerie and fed upon tuberculous meat, often become infected with tuberculosis, either pulmonary or visceral, whereas these animals never show spontaneous tuberculosis in the wild state. The dog becomes tuberculous when he swallows the sputum of his sick master; the calf, the cat, and the pig contract the disease when they are fed upon milk rich in the bacilli.

Insistence has rightly been laid, in recent times, upon the complete absence of tuberculosis in American pigs fed exclusively upon corn or other vegetable substances which may have been cooked, whereas this disease is extremely common in the pigs of our European farms, above all where the animals are fed upon the unpasteurized waste from dairies.

It is evident that tuberculosis thus developed—most often with primary lesions in the pleura or bronchial lymph-nodes—in pigs fed upon the milk of tuberculous cows, results from the absorption of the bacilli through the digestive tube. These ingested bacilli are able to penetrate the intestinal wall, and enter into the blood or lymphatic circulation, and are carried about in the body for a greater or less time before they produce the lesions characteristic of the tuberculous infection.

This fact was first demonstrated experimentally by Chauveau, in 1868 to 1872, and then by Villemin, Aufrecht, Gerlach, Klebs, Gunther and Harms, and a number of other observers, among whom one may mention Saint-Cyr, Viseur, Bollinger, Orth, Toussaint, Baumgarten, Rabinovitch, Parrot, Ravenel, Schroeder and Cotton, etc. Nevertheless, certain negative results attract attention, particularly those published by Colin (of Alfort) and by Moeller, which seem to demonstrate that

animals can swallow with safety large quantities of tuberculous material. We now know the reason of this. It is because artificial infection through the digestive tract is not invariable unless one takes certain precautions which I have definitely pointed out in studies with C. Guérin. It is necessary to cause absorption of the bacilli in a divided state so that they remain finely emulsified, as they are in the sputum or in milk. Under these conditions a single administration of infectious material is ordinarily sufficient to produce the tuberculous lesions, which in young animals most often remain localized for a longer or shorter time in the mesenteric lymph-nodes, but which in adults, on the contrary, appear at once in the lungs.

In studying the mechanism of the absorption of inert dust by the intestinal mucosa, I have been able to establish, with Vansteenberghe, that the same phenomena can be observed. The ingestion of finely powdered lampblack, or, better, of India ink, mixed with the food, produces in the adult guinea-pig the typical lesions of anthracosis of the lung, whereas in the young guinea-pig the colored granules remain for a longer or shorter time in the mesenteric nodes. Sections of the small intestine fixed during digestion allow one to recognize these colored granules surrounded by leucocytes in the chylous vessels of the villi.

In repeating our experiments upon this subject, Sir William Whitla[1] and Symmers have recently reached the same conclusions; and these workers have described an ingenious procedure which permits them to produce simultaneously tuberculosis and anthracosis of the lung or of the mesenteric nodes. This procedure consists in causing young or old guinea-pigs to swallow an emulsion of tubercle bacilli and India ink in olive oil.

Experimentation upon large animals such as cattle permits the determination with still greater certainty of the path which the tubercle bacilli follow to the lungs, if the animals are sacrificed, as in my experiments with Guérin, at successive intervals

[1] The Etiology of Pulmonary Tuberculosis. Cavendish Lecture, 1908. Lancet or British Medical Journal, July 11, 1908.

from the time of a single ingestion of the infectious material. One can thus convince himself, as has been shown by Chauveau, afterwards by Dobroklowski, that these bacilli penetrate the intestinal mucosa, even when this is entirely intact, and that they generally leave no trace of their passage. They are transported by the polynuclear leucocytes from the chylous vessels of the villi to the nearest mesenteric nodes.

In suckling animals and in young infants, they are frequently retained in the lymphatic organs which act as an almost perfect filter for the lymph. Sometimes they are destroyed in the nodes; sometimes they give rise to tuberculous lesions which, developing towards caseation, throw off their microbes into the efferent lymphatic canals, or occasionally into the peritoneum.

In older subjects, in whom the mesenteric nodes—as has been shown by Weigert—are much more permeable, the bacilli, always surrounded by polynuclear leucocytes, are carried with the lymph through the thoracic duct as far as the right ventricle of the heart, and thence forced into the capillaries of the lung. If the leucocytes carrying the bacteria have lost their ameboid movement because of the intoxication resulting from the tuberculin secreted by the bacilli, they are incapable of penetrating the walls of the capillaries by diapedesis, and they then give rise to fine emboli which become the starting point for a corresponding number of tuberculous lesions at the expense of the endothelial vascular walls (the gray granulations of Laennec).

The tuberculous lesions thus formed go on later to calcification or to caseation. In the second case, where the leucocytes do leave the capillaries, the tubercles drain into the alveoli or into some lymphatic vessel or vein, more rarely into an artery. They thus bring about a dissemination more or less rapid and more or less grave, of the virus into other parts of the body.

In my researches with Guérin, I have always been able to show the extreme frequency of adenitis of the tracheobronchial glands in young cattle, when the bacilli have penetrated the filter formed by the mesenteric nodes, and have reached the

lung. This adenitis is in constant relation with one or several tuberculous subpleural lesions which it is easy to discover.

The digestive origin of these lesions is entirely evident. We have reproduced them many times and Vallée (of Alfort) has also obtained them, either by feeding calves on milk proved to be from tuberculous cows, or by inoculating the bacilli directly into the mesenteric nodes after laparotomy.

Further, we have observed in some of our animals infected through the digestive tract, primary localizations of tuberculosis in other organs than the mesenteric nodes or the lungs. We have seen these appear under the form of pleurisy, of arthritis, of orchitis, and, in one very remarkable case in a young kid, of iritis. These exceptional localizations occurred only in animals infected by a single administration of small quantities of bacilli. One can suppose that these, because of their small number, had remained for a long time in the circulating blood, carried about by some polynuclear leucocytes, and that they had finally established a tuberculous lesion only in the organ where these leucocytes were when they succumbed.

Whatever interpretation may be accorded to these facts, it remains certain that so-called primary pulmonary tuberculosis and many other forms or localizations of tuberculous infection, manifestly result in a great number of cases from the penetration of the poison through the digestive tract.

Partisans of the theory that infections by inhalation predominate, above all Flügge in Germany and Kuss in France, bring forward the objection that in order to produce tuberculosis experimentally by ingestion, it is necessary to cause the animals to absorb thousands or millions of the bacilli, whereas a few individuals alone suffice to produce tuberculous lesions of the lung when they are inhaled. Those who think thus forget too often that among the millions of ingested bacilli there is only a small number—undoubtedly only a few individuals—which succeed in penetrating the intestinal mucous membrane, and that the greater part of those which break through are subsequently destroyed in the mesenteric nodes. Finally, there are only a few which are carried by the leucocytes as far as the

flow of lymph in the thoracic duct or as the capillaries of the lungs. But those bacteria which attain these situations establish the intravascular tuberculous formations so well described by Borrel, and later by Letulle, the slow development of which in successive steps finally gives rise to phthisis.

If so many physicians still persist in believing that man behaves differently from animals in the face of tuberculous infection, it is perhaps because the old ideas on *miasma* still weigh upon our brains. Without doubt, some among us have exaggerated the importance and the frequency of the infection of man by milk, and those who contend to-day that tuberculosis is contracted through the intestine more often than through the respiratory tract, bear the burden of these exaggerations.

We must protest also against this tendency to identify *intestinal origin* with *alimentary origin*. It is very certain that for our species the human patient is—I cannot repeat this with too much emphasis—the principal factor in the dissemination of tuberculosis. But I believe it necessary to declare strongly that while man can exceptionally contract tuberculosis, above all in infancy, by the inhalation of the bacilli, he contracts it much more often by intestinal absorption, repeated frequently for a long time, of some of these same bacilli freshly thrown off by a consumptive.

At the Congress of Veterinary Medicine at Cassel, on September 26, 1903, Von Behring advanced the theory that pulmonary tuberculosis of the adult might well be merely a tardy manifestation of a tuberculous infection contracted in infancy. He laid emphasis to support this opinion upon the frequency with which pulmonary lesions are observed in adult cattle, whereas in young cattle the mesenteric lesions are the most frequent.

My experiments with Guérin, and also those of Vallée (of Alfort) have shown the inaccuracy of so absolute a statement. We know to-day that a primary pulmonary tuberculosis can be produced in adult cattle, goats, monkeys, and guinea-pigs, by feeding these animals on infected material once or several times.

Since infection by the digestive tract is so easy, there is

reason for astonishment that tuberculosis is not a still more widely spread and deadly disease among live-stock than it is.

Now then, veterinarians and stock breeders have frequently stated that certain animals remain immune, although they have been in contact for years with animals suffering from the disease. More often still it happens that cattle react sharply to tuberculin at the first trial, cease to react a little later, and retain all the appearances of the most perfect health.

Must it then be admitted that in the first subjects the tuberculous poison has not been able to gain a foothold, and that the second are capable of complete recovery after a first attack? Here again, experimentation will enlighten us.

When we compel young calves to swallow, in a single contaminated meal, a small dose of tubercle bacilli of bovine origin, which have been very finely divided in order to facilitate their absorption, we notice that all these animals, without exception, contract tuberculosis. They react, on an average, thirty days later to the tuberculin test; and if we test them again each month following we find that after three, four, or five months, some of them cease to react. On being slaughtered these last present no tuberculous lesions, and if, having saved them, one attempts to reinfect them a short time after, by making them absorb a fresh dose of poison certainly capable of infecting other calves of the same age, one finds that they remain immune.

These animals then have really recovered from the primary lesions, and they should be considered as vaccinated, at least during a certain time the duration of which is still undecided.

On the other hand, if we administer to calves, not a single time but in several successive feedings, at close intervals, a series of small doses of the bacilli, not only do they never cease to react to the tuberculin, but we find that in them tuberculosis develops very quickly and becomes rapidly fatal.

It is then the animals which are exposed to a series of successive reinfections, so close together that they have not time to recover from the first attack, which become definitely and fatally tuberculous.

We understand from this why, under conditions moderately

predisposing to infection, certain subjects resist contagion for a longer or shorter time: it is because they have actually been vaccinated or rendered insusceptible by a previous attack, the lesions of which had time to heal before a new occasion for infection offered.

It is not easy to furnish proof that this state of immunity acquired by a previously successfully resisted attack exists also in man. Prolonged clinical observation of old cases, however, permits us to affirm that it is, at least in many cases, very probable. It appears especially evident in old cases of scrofulous lesions, and since the statements of Marfan in 1886, numerous doctors have described it.

One must inquire whether this is a question of a true immunity, of longer or shorter duration, supported not only by the absence of the reaction to tuberculin—which is not sufficient demonstration—but also by the non-persistence of the virulent bacilli in the different groups of lymph-nodes in the body.

The experiments which I have carried out upon this subject with Guérin demonstrate that at the end of the fourth month after the ingestion of the bacillary vaccine, a lymph-node is no longer virulent for the guinea-pig. The bacilli have then been absorbed and have completely disappeared.

We have attempted to prove the resistance of animals thus vaccinated through the digestive tract, with reference to an intravenous inoculation so serious as surely to produce in the control subjects the rapid development of an acute miliary tuberculosis with death in four to six weeks. This proof has been accomplished in six cattle, eight and twelve months after the administration of the vaccine. All the vaccinated animals were resistant and preserved the appearance of the most perfect health. But suddenly, about eight months later, one among them, although strict isolation was maintained so that infection from an outside source was impossible, manifested the first symptoms of a serious tuberculosis of the udder. All the others were then slaughtered; they did not react to tuberculin, nor did they show any visible tuberculous lesions, but their bronchial

and mediastinal lymph-nodes still contained living bacilli which were virulent when inoculated into guinea-pigs. The bacilli which had been injected intravenously, then, were not absorbed after eight months, whereas the bacilli which had previously been introduced by the digestive tract were not discoverable at the fourth month. And these bacilli remained latent in the body, not exciting any pathological trouble, up to the day when, the immunity ceasing, they became capable of suddenly producing disorders more or less grave.

From other experiments we are able to state that the cattle already suffering from benign tuberculous lesions and reacting to tuberculin, or that healthy cattle prepared by two or three large intravenous injections of tuberculin, manifest a resistance entirely exceptional to the severe tuberculous infections taking place through the veins. While the new subjects succumbed to the acute miliary tuberculosis in four to six weeks, the animals already tuberculous or prepared as I have above described contracted constantly a chronic form of tuberculosis with a very slow development. They showed, then, a resistance, incomparably superior to that of the healthy animals.

One observes the same phenomena in cattle artificially or spontaneously tuberculinized through the digestive tract, when one inoculates them later with a culture of tubercle bacilli subcutaneously. Thus Koch had already described in the tuberculous guinea-pig, at the time of his first work upon tuberculin, the formation of an abscess at the point of inoculation, but the neighboring nodes did not become infected, and the abscess healed when it opened on the surface.

Analogous facts are frequently established in clinical work on man. Every one knows that a local tuberculous suppuration occurring in a person with pulmonary tuberculosis, ameliorates the condition of the patient and considerably increases his resistance. Inversely, it is rare that patients in whom pulmonary tuberculosis has had a rapid development have been attacked previously by suppurations of the lymph-nodes, or bony or cutaneous tissues, except in the cases where an inopportune surgical operation has provoked an infection of the blood. It is a

well-known fact that about a quarter of the persons suffering from lupus present the auscultatory signs characteristic of pulmonary tuberculosis, and that these generally develop in them with very great slowness; likewise that many lupus patients live to an advanced age.

If one recalls that certain clinicians have pretended to obtain in phthisical patients real amelioration following the subcutaneous inoculation of cultures of virulent bovine tubercle bacilli (F. Klemperer), or of dead bacilli (Maragliano), or of cultures of human tubercle bacilli modified by passage through the body of a cold-blooded animal (crocodile) (Moeller) the experimental facts of which I have just spoken are of a nature to justify such assertions in a certain measure. But such a therapeutic method is certainly to be condemned; and so much the more because we possess in tuberculin a means as efficacious and less dangerous of attaining the same end.

Upon the whole, the resistance conferred by tuberculin and that which is observed in animals or in man already attacked by benign forms of tuberculosis (tuberculosis of the lymph-nodes or scrofula, tuberculosis of the bone, or of the skin, lupus) appear to be of the same nature as that which is artificially effected, whether by intravenous inoculation of human or bovine bacilli, following the methods of Behring or of Koch and Schültz, or by subcutaneous inoculation of the same bacilli (Lignières, Arloing), or by the insertion under the skin of collodion sacs containing cultures of human or bovine tuberculosis (Heymans).

In each of these cases it is not a question of true immunity, since the animals thus prepared, although not giving the tuberculin reaction, remain indefinitely carriers of living and virulent bacilli, and these are capable when the resistance begins to diminish of giving rise in the bodies of these same animals to serious lesions.

Let us recall, indeed, on the one side, that in the experiments of Melun (1906) on animals vaccinated with the "bovovaccin" of Behring, the inoculated bacilli were proved not to be absorbed at the end of six months (Vallée and Rossignol, Mossu), and,

on the other hand, that Roux and Vallée have demonstrated that vaccination intravenously or subcutaneously does not protect against infection.

On the contrary, the experiments which I have reported strongly support the statement that by the intestinal absorption of a weak and single dose of tubercle bacilli very finely divided, one can obtain at the same time the total absorption of the bacilli in the lymphatic system, and a state of immunity such that the animals are insusceptible, during a year at least, to large infections through the digestive tract.

Of course, we are not concerned here with a method of vaccination which one could think of employing in preserving the human race from this terrible scourge of tuberculosis. It would be rash to consider as very near a definite solution of this sort. But so difficult a study can proceed only by stages. Following Villemin and Robert Koch, who have laid the foundations upon which we build, a great number of research workers have brought their stones. Others will follow, and the work will be accomplished for the glory and for the salvation of humanity.

FEVER*

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FROM the earliest times fever with all its remarkable symptoms has been familiar not only to physicians but even to the general public, and the term is one so honored by age and by the good that must consequently inhere in it, that it cannot be discarded or dismembered. It is, nevertheless, very difficult to define this conception clearly because even yet we are unable to say with certainty at what point the direct effects of the cause of the disease end and what really belong to the fever. For, although the elevation of the body temperature is one of the most salient points, it is by no means the only characteristic nor is it itself always to be regarded as an infallible sign of fever, for such elevation of temperature may occur in a perfectly healthy person, if, for example, he be immersed in a hot bath. There are other readily recognizable signs of fever, such as thirst, and weakness, and alterations in the character of the urine, but it is difficult, indeed, to be sure what part of each of these is produced by the bacterial poisons that cause the disease, what part by the fever itself. Kraus,[1] in his recent review, has, on this account, written of fever and infection together.

It is clear, from the fact that the ideas that prevail as to its general character are so uniform throughout the world, that, no matter what the nature of the disease that brings fever with it, the fever itself is the same. Sometimes it is ushered in with a chill, sometimes it begins gradually; it may be constant or intermittent; it may end abruptly or slowly disappear—but always it is recognizable as fever. Therefore, it seems proper that we should speak of it as the febrile reaction—as something

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characteristic of the body and not of the disease, and it is from this point of view that I shall consider it. The significance of this reaction I shall discuss later after reviewing the facts concerning the phenomena which are peculiar to it and the upheaval in the whole economy of the body which accompanies it. We shall be particularly interested in comparing it with other reactions, for it is evident to the most casual observer that nearly everything that affects the body at all is responded to by some sort of reaction, and that most of these are processes which have been evolved in order to maintain the life and health of the individual. For example, the swallowing of food is followed by the most complicated reactions—muscular movements comminute it, ferments are secreted to digest it, changes in the whole metabolism follow its absorption, and so on. Exposure to heat or cold, hunger or thirst brings into play protective reactions which regulate the body temperature or cut off the lavish expenditure of foodstuff and water still stored in the body so as to protect and prolong life as far as possible. Indeed, it would be difficult to produce such a state of repose that none of these responses would be in progress.

TEMPERATURE REGULATION.

Perhaps the most striking characteristic of fever is the elevation of the body temperature above the normal. The fact that in certain classes of animals the temperature of the body is maintained nearly at a fixed level is in itself a matter to arouse our wonder and interest, and the reason for this regulation of the temperature above that of the surrounding atmosphere (for even in the tropics the average temperature is far below that of the body), and for the constancy of its level would afford much material for discussion. With the exception of mammals and birds, all animals seem to be devoid of any such mechanism and are in consequence poikilothermic.[2] The temperature of their bodies, like that of inanimate substances, quickly adapts itself to that of the surroundings, and heat produced in the course of their metabolic processes is at once dissipated, the more rapidly the smaller the animal on account of its

relatively greater radiating surface. In mammals and birds, however, there are special arrangements for preventing such loss of heat, or under other circumstances of facilitating it. Thus the development of the sweat glands in some animals affords them an especially effective method of cooling off the body, while the too great dissipation of heat is prevented in others by the thick layer of subcutaneous fat or the covering of hair or feathers. Man alone finds it necessary to resort to artificial modifications of the temperature of the air and to artificial protection in the form of clothing. It may well be a matter of speculation as to whether after all, it was not this need of artificial heat regulation which brought about our suddenly acquired knowledge of good and evil rather than the reverse, as is so generally conceded.

Different animals have, of course, different standards of temperature and even for one species there are slight individual variations; nor is the temperature of various parts of the body the same, largely because certain portions are more protected from loss of heat than others, but partly also because, as it seems, heat is more abundantly produced in certain organs than in others. This point may be discussed more advantageously later, but here it may be said that this inequality in local heat production is continuously compensated by the rapid heating of the blood which comes into such close contact with these tissues and then hurries away to warm others.

Although the variations are relatively slight, even warm-blooded animals are subject to changes of a periodic character in their temperature. This is particularly noticeable in birds, in which there is a considerable fall of temperature during the night. I have observed that in normal crows the curve of the temperature, when taken every three hours, shows a regular daily variation of 3° F. or more, the minimum temperature of 104.5° F. being recorded at midnight or at 3 A.M., while the maximum of 108° F. is reached at 3 o'clock in the afternoon.[3] To a certain extent, then, the crow must be regarded as poikilothermic. But even in human beings a similar periodic fluctua-

tion of not more than 1.5° C. is observed and has been recorded in the form of curves by Jürgensen,[4] Benedict and Snell,[5] and others. In these curves, as in the case of birds, the lowest temperature occurs in the early morning hours, while the maximum is reached in the afternoon. In addition to the effects of bodily activity of all sorts during the day, it seems possible that there may be causes of a more fundamental character, perhaps even a rhythmicity in the regulation of the temperature, which maintains this uniform curve.

That the temperature of the body is not kept at a perfectly constant level is shown further in the temporary alterations which can result from outward influences and the exercise of bodily functions in the normal individual. Slight elevation of the temperature may follow the taking and digestion of food, and muscular work, if violent enough, may result in a distinct rise in temperature. Ordinarily, however, in freely moving normal animals, such changes are insignificant and compensated at once by the regulating mechanism, and if we observe a marked elevation of temperature in the course of violent muscular activity, it is probably because the normal compensatory dissipation of heat can not take place rapidly enough to keep the body cool. Interesting examples of this are found in tetanus and the status epilepticus, in which very high temperatures are sometimes observed. I had the opportunity recently of following the temperature in a dog in which after parathyroidectomy the most violent tetany developed with intense spasmodic muscular contractions affecting the whole body. The temperature, which had been 39° C., reached 43.2° C. during this attack, in spite of the fact that the respiration had become extremely rapid (300 to the minute). The administration of calcium acetate stopped the convulsions in a few minutes, and within half an hour the temperature dropped to 38.9° C.

Exposure to excessive heat or cold does not, as a rule, alter the temperature of the normal animal unless the exposure is so protracted and the difference in temperature so great that the mechanism of heat regulation finally becomes inadequate.

At a certain limit the control is overpowered and the animal's temperature rises or sinks, as the case may be.

These exceptions to the normal maintenance of a practically constant body temperature, unimportant and transient as they are for the greater part, I have mentioned in order to emphasize the wonderful efficacy of the mechanism of heat regulation. A few words will recall to mind the general character of this mechanism which has at its disposal means for the generation of heat as well as contrivances for the rapid dissipation of the heat produced or for its retention and husbanding within the body. The control of these matters has been clearly shown to reside in the brain, for the severance of nervous connection with the higher parts of the cerebrospinal system leaves the body in the condition of a poikilothermic animal. Whether this is due, as Tigerstedt [6] tends to believe, to the bathing of the brain with blood too hot or too cold or to the transmission of sensations of heat or cold by the nerves, it is difficult to say with certainty.* Nor are we satisfactorily informed as to the precise portion of the brain which is concerned in this process of heat regulation, although the discovery of many so-called thermoregulatory centres has been reported. That one found by Aronsohn and Sachs [7] in the anterior medial portion of the corpus striatum has been most generally accepted, and many investigators have confirmed their statement that the puncture of this area will cause an elevation of temperature which lasts several days. This sort of hyperthermy has been studied a great deal in connection with fever, and we shall refer to it frequently.

The generation of heat can be effected by the nervous system in the musculature either by the production of actual movements, including such as are evident in shivering, or by the heightening of the muscle tonus. Whether the brain by send-

* Of great interest in this connection would be the accurate study of heat regulation in patients suffering from syringomyelia in whom all appreciation of heat and cold is lost. Except for the existence of disturbances of the vasomotors of the skin and the sweat secretion, I can find no satisfactory evidence on this point.

ing out impulses of any sort to the muscles or to the other tissues can directly accelerate or intensify the chemical processes which lead to the production of heat is still questionable.

The regulation of the dissipation of heat, on the other hand, is very directly controlled by the nervous system, and the mechanism employed is adjusted with great delicacy. Thus radiation and conduction of heat from the surface of the skin depends on the calibre of the cutaneous blood-vessels, which can be changed within very wide limits by the vasomotor nerves; in order to keep the body from becoming too warm, whether from excessive production of heat or from the high temperature of the surrounding medium, the skin is flushed with blood, while exposure to cold is quickly followed by such contraction of those vessels that the body surface becomes very pale and the warm blood is restricted as much as possible to the interior and protected from cooling. Even more effective is the secretion of sweat, the evaporation of which keeps the body cool even when exposed to very high temperatures. In all animals, but especially in those which possess no sweat glands, a similar result is attained by the exhalation and evaporation of water from the lungs, and it is well known that in such animals as the dog this is an extremely important method of eliminating heat, the evaporation being greatly increased by acceleration of the respiration. If, as in the experiments of H. Winternitz,[8] the mechanism for the dissipation of heat can be incapacitated by immersing the person in a hot bath at a temperature above the normal body temperature, a curious change occurs in the respiration. Apparently there is no mechanism in the human being to respond to such circumstances by accelerating the respiration, but it becomes greatly increased in volume, possibly in the attempt to compensate for the usual cooling effect of sweating. In dogs so immersed, on the other hand, the rate of respiration increases from about 15 to 300 or more, for the dog still has his normal method of cooling himself.

In such experiments the body temperature and heat production as measured by the chemical changes are greatly elevated and may here be regarded as escaping from the control of the

regulating mechanism. On the other hand, if the animal is exposed to cold, as long as its heat-regulating mechanism retains its control and the body temperature remains constant, the heat production as measured by the chemical changes is also increased, but sinks when the regulation is no longer maintained and the temperature falls.[9 10]

HEAT PRODUCTION AND LOSS IN FEVER.

If we now turn our attention to the condition of the temperature, heat production and loss, in fever, we find that so much light has been shed on the subject in the past few years that we may speak of certain questions with some degree of security, although there remain many points which are still obscure.

It is evident that there are several possible ways in which the temperature of the body may be elevated. The heat production may increase while the heat loss remains constant, or, the heat production remaining constant, the heat loss may be diminished, or both may be elevated but not proportionately. Any disproportion, however slight, between the two which leaves a positive balance of heat will in time bring about an elevation of the body temperature. In this sense we are reminded of the wonderful symmetry of action of the two sides of the heart. The slightest continued disproportion between the output of the two ventricles such that the left ventricle ejects less than the right will in a short time lead to the enormous overdistention of the pulmonary vessels and œdema of the lungs. It is plain, therefore, that a great increase in the production of heat is by no means unconditionally necessary for the elevation of the body temperature, and we can readily understand that the continued accumulation of slight excess may quite rapidly lead to a very striking pyrexia.

Traube attempted to explain fever on the basis of the second alternative mentioned—temperature elevation from excessive retention of heat alone, and supported his theory ingeniously, but it needed only the determination of any increased heat production in cases of fever to shatter this theory. And this

proof has been brought by many workers, chiefly by means of a study of the respiratory gaseous exchanges, which showed that oxidation and consequent heat produced is increased, but also by the direct measurement of the heat produced by an animal during fever, a measurement which can be carried out by the aid of a suitable calorimeter (direct calorimetry).

It is important to observe that the more recent writers on this subject estimate the increase in the heat production during fever at a much lower figure than did the earlier observers (Senator, Finkler, Colasanti, Lilienfeld and others), who often found an increase of 75 per cent. or even more. With Krauss [11] there began a series of more accurate investigations, in which stress was laid on the importance of keeping the animal under observation in a state of muscular repose, since he recognized that the earlier work was untrustworthy on account of the great variations produced by muscular contractions, shivering, etc. After eliminating these sources of error, he found the febrile increase in heat production to be represented by an increase in oxidation of 20 per cent. at the utmost. Löwy [12] also found the increase in combustion relatively slight, sometimes sinking within the limits of normal and at the highest rising to 51.8 per cent.

Nebelthau,[13] recognizing the inaccuracy of this indirect method of estimating the heat production when applied for short periods, studied the heat production and loss in febrile rabbits by direct measurement in a calorimeter, the observations being made to extend over a long period. He, too, found a distinct increase in the output of heat, but thought it still conceivable that fever might arise from the coexistence of a constant heat production and diminished heat loss.

Krehl and Matthes [14] found an increase in heat production of 14 to 60 per cent. in the height of certain fevers, while Staehelin [15] in his febrile dog observed at first a diminished heat production, although later in the height of the fever the normal was exceeded by 45 to 47 per cent. May [16] in febrile rabbits similarly found a normal heat production on the first day of the fever, but a rise of 5 to 28 per cent. on the second day.

All these authors agree, therefore, that in fever there is a distinct increase in the heat production. When they speak of an increase of 25 to 50 per cent. in the heat production as compared with the normal it seems at first sight an enormous change. The true significance of this statement is not clear, however, until we examine the heat production under some other conditions, and a hint of the relative importance of the influence of these other conditions is given by the refusal of the

recent workers to accept the results of their predecessors because they had not carefully attended to the exclusion of the effects of chance muscular movements during the observations. Speck [17] even goes so far as to assert that there is no increased heat production in fever, that even the modern figures are a delusion based on the overaction of the respiratory muscles, the intensification of the heart beat, etc.

The amount of heat produced in the body on the ingestion and absorption of a full meal is very greatly increased over that produced by the same person when at rest and with an empty stomach. Many earlier workers have demonstrated this, and Staehelin,[18] in his recent careful study, has shown very clearly the effect of various forms of food, the increased energy production being greatest after a meal composed of carbohydrate and proteid. Such an increase in heat production may surpass by a great deal that observed in fever, but there need be no rise in temperature. With muscular work the increased heat production is enormously greater. In contrast with the febrile patient at rest, in whom, as we have seen, heat production reaches a moderate excess, the normal man, in the course of muscular labor, produces an amount of heat often several hundred per cent. in excess of that developed while he is at rest —and still there need be no elevation of his body temperature. He differs from the febrile patient in the extraordinarily rapid discharge of heat by all the means at his disposal.

Although it is obvious from the direct calorimetric estimations mentioned that the heat loss is increased during fever as well as the heat production one must gather, from the knowledge of the normal methods of heat regulation during great elevations of the heat production, that in fever the dissipation of heat is relatively restricted, and that, even though the body has not at its disposal the enormous quantity of heat produced by the well-fed and working man, the metabolism is somewhat accelerated and some of the excessive heat is retained. This is sufficiently clear, although I can not find perfectly precise measurements of the amount of heat produced during a certain period contrasted with the amount lost in the same period in

order to show concretely the direct cause of the elevation of temperature.

Liebermeister,[19] as is well known, entertained the view that the regulating mechanism is in fever peculiarly altered to react for a different standard of body temperature. As he expresses it, the regulating mechanism is tuned up to a higher pitch so that it begins to allow of the escape of heat only at a higher level, exactly as we might screw up the thermoregulator of a thermostat so that its temperature would stand at 40 instead of 35. There is something very attractive about this idea, but it must be said that the experiments which might establish it as a fact have not been carried out satisfactorily.* In general it is known that muscular effort during fever may drive the temperature up—that the taking of food may elevate the temperature, and that even after convalescence has begun the temperature regulation of a febrile patient is very labile and easily disturbed. But we do not know precisely whether the output of heat produced by muscular work during fever would be regulated at this higher standard in a way resembling that in the normal. If Liebermeister's theory is to hold, the heat produced from muscular work should be dissipated after the requisite amount to maintain the heightened body temperature has been accounted for, in the same way as in the normal.

Much interest is attached to the variations in heat production and loss, and the consequent temperature in the various stages of fever. It seems probable that this must vary in dif-

*Efforts have been made by Stern, Filehne and others to show that there is some such constancy in the activities of the heat regulating mechanism, but Krehl points out, very justly as it seems, the fact that there is rather a weakening of the control so that febrile patients are more subject to external influences than normal persons—they are more easily cooled in a cold bath and more subject to the effects of antipyretics. Of course, the abnormal condition of the cutaneous vessels and other instruments of the regulatory mechanism must be remembered, but the very inconstancy of the body temperature during fever seems to him enough to indicate that here we are not dealing with an adequate regulating mechanism which is merely tuned to a higher pitch, but rather with one which to a certain extent has lost its control.

ferent types of fever, for the characters of these stages differ so much, but it is certain that in the initial stage, especially in those cases in which fever is ushered in by the appearance of a chill, every mechanism is set to work by the body to limit, as far as possible, the escape of heat and by increasing at the same time the heat production to elevate the temperature as rapidly as possible. The skin becomes livid and blue and cold as the result of the energetic contraction of the cutaneous vessels; the very important methods of heat loss, radiation and conduction, are thereby restricted to the maximum degree. Evaporation from the skin and from the lungs is also decreased, although in some cases that from the lungs has been observed to be heightened. Further, the patient, from the very sensation of cold produced by these changes, draws his body into the smallest compass and covers himself thickly with clothes. On the other hand, heat production is greatly increased, and especially so by the increased tension of the muscles, by the shivering, and even by the goose flesh. Liebermeister found an increase of 21 to 24 per cent. in the heat production in the hot stage of a malarial attack, in the chill 147 per cent. increase. Naturally the muscular contractions are chiefly responsible for this high figure. Krehl and Matthes [20] find that in this stage both heat production and heat loss fluctuate a great deal, but that they diverge from one another far more than in the normal.

During the fastigium or height of the fever the disproportion between heat production and heat loss is less striking. Conduction and radiation of heat from the skin and evaporation approach the condition found in the normal person on the same diet [21] and in the same surroundings, but this in itself is abnormal, for in a healthy person with such an elevated heat production these regulating mechanisms would be greatly stimulated.

The blood supply to the skin in the height of fever undergoes remarkable fluctuations, as Senator [22] pointed out; the dilatation and contraction of the vessels may vary from hour to hour and even from one part of the skin surface to another, and consequently it seems probable that if we could determine

the heat loss from the skin for very short periods we would obtain a curve showing very marked irregularities. In this stage, then, we have an irregular and moderate increase in the heat production associated with an irregular increase in the heat loss. However much the heat production may increase, the heat loss keeps a place somewhere behind it, and there is no parallelism between the temperature and the heat production or between temperature and the amount of oxidation going on. Krehl and Matthes point out the fact that the special cause of the fever does not necessarily govern the intensity of the heat production, over which the nutritive condition of the patient and the individual type of reaction have a great influence. The young strong patient tends to show a higher heat production than does the aged, weak one, and it is a commonly appreciated fact that the lack of an intense febrile reaction, like the absence of adequate leucocytosis, is often an index of the low powers of resistance of the patient. When a patient is overcome by the intensity of the infection, it is also usual to observe a fall of body temperature to a subnormal level.

In the last stage of fever, that of defervescence, there is a gradual or sudden strengthening of the mechanical dissipation of heat and at the same time a decrease in the heat production. One receives the impression that the mechanism of heat elimination, having been held in leash by the regulating nervous system, is now set free when the poisoning is overcome and the saving of heat no longer necessary. Especially in the critical forms of defervescence is this outbreak of heat dissipation seen to advantage, merely because there the phenomena are concentrated into a short period of time.

TOPOGRAPHY AND MECHANISM OF FEBRILE HEAT PRODUCTION.

Since I wish to discuss in some detail the changes in metabolism which underlie these disturbances of the heat economy of the body, the limits of this lecture will not allow me to enter on a consideration of the topography of heat production further than to say that the muscles are commonly regarded as the great source of heat, although, of course, every tissue is concerned to some extent in its development. In fever there

are, as we shall learn, certain special reasons for thinking that heat production is brought about chiefly in the muscles. There are, however, those who disagree with this assumption and believe that the liver is even more prominent in this connection. Hirsch and Rolly [23] find that after the influence of the muscles is destroyed by curare, it is still possible to produce hyperthermy by heat puncture, and, since this is not successful in glycogen-free animals, they ascribe the production of heat to the combustion of carbohydrates in the liver, and by thermo-electric methods show that it is only after the temperature rises in the liver that it does so in the muscles. Hirsch and Müller [24] apply the same thermometric and thermo-electric methods to the mapping out of the heat production in the body, and find in fever that the liver is far warmer than the muscles, and that even the subcutaneous tissues have a higher temperature. They regard the low temperature of the muscles as notable, and hold that Heidenhain's view that the muscles are warmer than the aortic blood is thus disproved. The methods employed in these researches seem not entirely above criticism, for we have again a conclusion as to heat production based on the temperature of an organ, and, in my judgment, we must still defer our ultimate conclusion as to the relative importance of the muscles until further work is brought out.

Nor can I consider at length the interesting discussion as to the claims of the theories of the neurogenic and toxogenic nature of fever. The precise character of the nervous mechanism which presides over the heat economy of the body is far from being well understood, but I have already mentioned the fact that there are certain localities in the brain, irritation or injury of which causes hyperthermy, and have drawn especial attention to that so-called heat centre which was discovered by Aronsohn and Sachs in the anterior medial portion of the corpus striatum. The character of its action is, however, not understood; we are far from possessing definite proof of the existence of any special nerves which preside over the production of heat, and it is at this point that the neurogenic theory of fever is attacked. Hirsch, Müller and Rolly [25] put forward the idea that it is not from the action of the nervous system that the

febrile intensification of metabolism arises, but rather from the introduction of poisonous substances which directly injure the cells. While the neurogenic chemical heat regulation occurs in the muscles with the increased burning of carbohydrate, the increased burning in fever is probably universal and affects especially the proteids, the part played by each organ depending on the intensity of its specific function in metabolism. Fever is thus a specific reaction against injurious materials which affect the tissues. Krehl and Soetbeer [26] find that in cold-blooded animals, such as the frog, infection results in a marked heat production and dissipation; and, inasmuch as these animals have no nervous mechanism controlling heat regulation, they see in this result a proof of the toxogenic theory of fever.

It would seem perfectly possible to control the part played by the nervous system in fever by a variety of experiments. No one doubts the dependence on the nervous system of the mechanism governing the dissipation of heat, and the question is rather as to the relation of the heat production to the superior nerve elements. Of course, it is plain that by causing muscular contraction or increasing muscular tone the motor nerves can increase heat production, and one might be inclined to say that in all probability this is the way in which the heat production is stimulated in all cases. But it is frequently observed that when the spinal cord high in the neck is severed there occurs a febrile rise in temperature with increased heat production,[27] although often it is the reverse, a gradual sinking of temperature. Section of the medulla near the pons causes a rise in temperature, and the injury of the corpus striatum spoken of above very regularly brings this about. Analysis of such effects shows that the elevation of temperature is not due solely to limitation of heat dissipation, for Schultze [28] finds that animals with heat puncture react to outward changes in temperature precisely as do normal animals. There is, on the contrary, an actual increase in the production of heat, even though the animals remain perfectly quiet, and, although there has been some disagreement as to the actual source of the heat in these cases, it seems probable that we must accept the results of Senator and Richter,[29] who find that there is not merely an

increased combustion of carbohydrates, but that proteid metabolism is also increased. In no essential particular, then, does this hyperthermy differ from that of fever.

Despite all this evidence that a somewhat peculiar febrile reaction can be produced by injury of the brain and despite the perfectly certain and generally accepted fact of the nervous control of the heat-dissipating apparatus, I feel that there is much to be said for the view that the circulating poisons affect directly all the cells of the body, and that the latter in their quality of living protoplasmic creatures react with an increased metabolism. It appeals to me as a reaction quite analogous to that in which leucocytes are produced in enormous numbers in the far-off bone-marrow when they are needed in the pneumonic lung, and is no more easily explicable than this.

METABOLISM IN FEVER.

Having discussed the general subject of heat regulation in connection with fever, in some of its aspects, we may now turn to the consideration in somewhat greater detail of the chemical processes which underlie the production of heat. It may be said in general that these processes, in so far as they are connected with heat production, consist chiefly in the combination of oxygen with carbon and with hydrogen with the formation of carbon dioxide and water. That this outcome may be interrupted by the formation of intermediate products detracts in no way from the truth of this statement, and if only sufficient time be devoted to observation the final results are the same. That there are other oxidations going on is, of course, true, but their rôle in comparison with those mentioned is almost negligible. Further, the amount of heat produced in the various decompositions and syntheses is very slight, and we shall not go far astray in restricting our attention to the oxidation.

Something has already been said concerning these processes in discussing the production of heat in so far as the amount of heat produced in fever was compared with that produced in other conditions. Now, however, it is important that we should be able to compare more accurately the figures which result from

the estimations of oxygen in varying conditions, and at the risk of becoming tedious I shall quote the statements of various authors as to the total oxidation products in various stages of hunger and nutrition, in muscular work, in exposure to heat and to cold and finally in fever. Naturally these results may vary widely because of the size and characteristics of the individual observed and because of difference in the character of the nutrition, but they will doubtless diverge so far from the normal that there can be no question as to the effects of the conditions which they are meant to illustrate. For the sake of convenience, they may be set down in the form of a table.

TABLE SHOWING STATEMENTS OF VARIOUS AUTHORS AS TO OXIDATION PRODUCTS IN VARIOUS CONDITIONS.

Subject	Condition	CO_2 c.c. per min.	Output Gm. per hour	Oxygen c.c. per min.	Absorption Gm. per hour	Author
Healthy Man—						
In bed, fasting. Especially quiet			21.4			Johannson[30]
Ordinarily quiet			28.1			
Healthy Man—						
Fasting			36.5			Johannson[31]
During and after meal			41.5–49			
Sleep			30			
After five days' hunger.						
At rest or reading			24–29			
During and after a walk			30			
Sleep			18			
Healthy Man—						
Exposed to cold and heat.						Volt[32]
Surrounding temp., 4.4° C.			35.16			
Surrounding temp., 15.2° C.			26.33			
Surrounding temp., 30° C.			28.5			
Healthy Man—						
Rest			27–37			
Walking			49–67 (increase 13–45)			
Climbing			86–131 (increase 50–104)			

Subject	Condition	CO_2 c.c. per min.	Output Gm. per hour	Oxygen c.c. per min.	Absorption Gm. per hour	Author
Healthy Man 67.2 kg.—						
1.	Complete repose. Fasting		19.86		17.45	Staehelin[56]
2.	Complete repose. Proteid meal, 75 Gm. proteid		23.50		22.54	
3.	Complete repose. Fat meal, 7 Gm. proteid, 77.4 fat, 30 carbohydrate		22.65		21.88	
4.	Complete repose. Carbohydrate meal, 32 Gm. proteid and carbohydrate, 189 fat		24.08		21.41	Staehelin[54]
5.	Complete repose. Proteid and carbohydrate, 90 Gm. proteid, 34 Gm. fat, 113 Gm. carbohydrate		25.80		23.61	
Healthy Man, 48 kg.—						
	At rest. Temp. 36.2....	133.8	15.86	172.9	14.38	Winternitz[58]
	After 35 min. in bath at 40–41 C. Temp. of pt. 38 C.	316.2	37.50	363.6	31.18	
Woman, 61 kg.—						
	Erysipelas. Temp. 40 C...	303.91	36.02	321.41	27.57	Kraus[55]
	Fever-free one month later	224.68	25.64	245.18	21.3	
Man, 48.5 kg.—						
	Typhoid, 2nd week. Temp. 39–40.8	284.12	33.69	350.4	30.02	
Man, 48.5 kg.—						
	Pneumonia. Temp. 38.9–40.5	234.9	27.85	25.56	21.91	
Man, 58 kg.—						
	Pleuritis. Temp. 39.5....	254.78	30.21	315.1	27.02	Löwy[57]
Man—						
	Typhoid. Temp. 39.5....	263.99	31.30	398.32	34.16	
Man, 50 kg.—						
	Typhoid. Temp. 39.5....	205.79	24.40	291.72	25.02	
Man, 50.5 kg.—						
	Tuberculosis. Temp. normal	206.29	24.43	255.95	21.95	
	Tuberculin injection. Temp. 40.2	280.96	33.12	316.25	27.15	

Subject	Condition	CO_2 c.c. per min.	Output Gm. per hour	Oxygen c.c. per min.	Absorption Gm. per hour	Author
Woman, 61.4 kg.—						
	Suspected tuberculosis.					
	36–37	289.51	34.22	381.41	32.71	Kraus and Chvostek[28]
	Tuberculin injection. Temp.					
	39.2	310.84	36.86	417.20	35.78	
Woman, 46 kg.—						
	Pulmonary tuberculosis.					
	Temp. 37.9	184.15	21.83	268.07	22.99	
	Tuberculin injection. Temp.					
	40.2	258.97	30.71	399.17	34.32	

This table suffices to show that in fever in man the oxidation is usually but not necessarily increased, but that the increase is not very great. Kraus estimates it as not exceeding 20 per cent. at the highest, while in some cases it is not elevated at all. And Löwy, Riethus and others concur with him in this opinion, believing that the higher percentages given by the earlier workers (Senator 75 per cent.) were probably dependent on the muscular unrest which prevailed during their observations. Similar results have been obtained in the case of animals in which fever has been experimentally produced, and I need only refer here to the careful studies of May[39] and Staehelin.[40]

May gives the result in one of his hungering rabbits in which he produced fever by inoculation with the bacillus of swine erysipelas as follows:

Rabbit E	CO_2 output	Oxygen absorption	Resp. quotient
Before infection	48.756	46.861	0.7567
After infection and during fever	53.035	50.700	0.6953

Here we have an increase in the carbon dioxide of only a little more than 8 per cent., and Staehelin in observing the dog infected with surra found the following changes in the carbon dioxide output:

	Carbon dioxide output per day (aver. of several days)
Before infection	178.6
After infection—First period. Temp. 37.9–39.4	169.85
Second period. Temp. 39.5–40.1	206.75
Third period. Temp. (?)	229.0
Fourth period. Temp. 38.5–40.4	195.5

The maximum increase of carbon dioxide in this case is 28 per cent.

It is obvious that the value of such estimations of the total oxidation in fever lies in the fact that we can thus compare roughly the extent of the oxidation in that condition with that

observed in a normal person, or in a person doing muscular work, and can judge as to whether the metabolic changes are in gross greater or less than in those persons. We may even compute, if we know the total nitrogen output, the proportion of this total oxidation which pertains to the burning of nitrogenous materials and that which results from the combustion of the nitrogen-free substances; but, unless we know more than this, we can gain no very accurate idea as to the more intimate nature of the processes which go on in fever as contrasted with those in a healthy person. It is known, for example, that in the healthy person, as long as there is a sufficient supply of energy-producing food, the tissues themselves are not attacked; it is known, further, that the oxidation in the normal person is very much modified by the character of the food, and, still further, that in muscular work in the normal individual the oxidation associated with the supply of energy for the work does not affect even the circulating proteins, so long as there is abundant supply of non-nitrogeneous materials. These things are, however, by no means to be assumed as true in the case of the febrile person. We must, therefore, in order to determine the exact nature of the processes of oxidation in such a person, even more than in the normal, make such studies as will give us evidence of the exact amount of protein, of carbohydrate and of fat in the food; we must know the amount of water taken into the body and, if possible, the amount of oxygen absorbed. We must then know the output of these various substances, preferably in health and in fever in the same individual, and this information must include at least the amount of nitrogen excreted in the urine and fæces, and the amount of carbon in the urine, fæces and respiration as well as the amount of water excreted by these channels. We can then, and then only, determine the balance of nitrogen, carbon and water; and in order to estimate with any accuracy the suspected changes in the tissues such information as to the balance seems necessary. Even then the methods which have been mentioned for the appraising of the amounts of the non-nitrogeneous substances oxidized are perhaps not absolutely to be relied on, for unless

the animal is starved until it can be regarded as glycogen-free—always a doubtful assumption—we must depend on the idea that after the amount of carbon dioxide corresponding to the nitrogen excretion is deducted the remainder is to be applied, first, to accounting for the oxidation of carbohydrate and only after that the oxidation of the fat. Further, the existence of the intermediate products of oxidation can not be suspected unless we know the respiratory quotient; that is, the relation of the output of carbon dioxide to the absorption of oxygen.

The exact estimation of the nitrogen balance seems to be of especial importance in the study of the protein changes in fever which are so prominent. In the literature of the subject, however, I can find no such studies of fever in man; nearly all—I may say all—are fragmentary in the sense that the character of the food is not recorded or that the estimations of excreta are not sufficiently complete to enable one to do more than infer from comparison with approximately similar estimations, in the normal, the nature and extent of the change. Naturally only divergences of considerable extent from the average normal will be recognized in this way. The recent researches of Staehelin on the metabolism of non-febrile tuberculous patients give, however, a very good model for such work.

Nevertheless, the principles involved are rendered clear in the papers already mentioned of May and Staehelin.

May starved his rabbits until a uniform nitrogen output was established and then inoculated them with the bacillus of swine erysipelas and was able to draw up such tables as the following, in which, since the rabbit received no food, he could accurately assign the oxidation products to the body tissues from which they sprang:

Temperature of animal	Nitrogen of urine	Carbon			
		Respiratory carbon	From proteins	From non-nitrogenous materials	
39.5....	1.30				
39.2....	1.45				
39.5....	1.79	13.22	5.796	8.848	
39.7–41.2....	1.81	13.10	5.864	8.675	
40.8–40.7....	2.45	14.53	7.938	8.545	
34.7–32.5....	0.103	12.337			Collapse

Staehelin fed his dog but estimated precisely the constituents of the food and the consequent nitrogen and carbon balance in the excreta. From this, since the deficit of nitrogen and carbon showed that there was constant tissue destruction, he could feel sure that the increased output of carbon was derived from body protein and body fat and could estimate the proportion in which they were oxidized. His tables * show very plainly that there is not only a rapidly increasing protein destruction during the fever but that there is also an increasing oxidation of body fat, which is far too great to be ascribed to inanition. To this relation of the fat it will be necessary to return. Staehelin summarizes the total body destruction of the animal in this experiment as follows:

	Protein destruction	Fat destruction
Preliminary period	8	51
Inoculation and prodromal	17	73
First period of fever	29	49
Second period of fever	110	204
Third period of fever	47	79
Final period	143	175

The value of the respiratory quotient which gives us a clue as to the qualitative character of the oxidation processes does not seem to have been as great in the case of fever as might have been expected, for there is still much dispute as to its amount in that condition. The earlier authors found, as a rule, that this co-efficient was lowered in fever—thus Regnard, Riethus, Finkler, and Löwy all report such a diminished respiratory quotient. Senator sums up his results in the following statements:

Even in the most favorable case the increase in the carbon dioxide output is far less than the increase in the urea excretion. In the febrile body there is not (unless perhaps in the earliest hours of the fever) any abnormal accumulation of carbonic acid—rather the reverse. Therefore, in fever the burning of nitrogen-free carbonaceous material (fat) to its end product (carbon dioxide) can not occur to the same extent as the decomposition of protein into urea. On the contrary, there seems to be rather a decrease of fat combustion than an increase, and the fevered animal will therefore become relatively richer in fat than a non-febrile animal in the same condition of nutrition. This probably explains the fatty degeneration which occurs in the tissues in febrile diseases.

* Staehelin's tables are too long to reproduce here, but they have been copied by Kraus in Von Noorden's Metabolism and Practical Medicine, Volume II, where they are perhaps more accessible than in the original paper."

Kraus, in 1891, found that no direct relation existed between the extent of oxidation and the degree of pyrexia and concluded that any qualitative alteration of febrile metabolism that might be found was insufficient to influence the respiratory co-efficient in a recognizable degree. As a matter of fact, he found the quotient practically unchanged and thought that any changes which might occur were determined by the existing nutritional state, by the bodily condition and by the material available for metabolism. Jaquet has adopted the same view and has shown that a diminution of carbon dioxide elimination in the respired air may depend on a diminished respiratory effort and less complete ventilation of the lung.

Staehelin gives the following figures from his experiments (average in round numbers):

	Respiratory quotient
Normal person, fasting	0.82
Normal person, after protein meal	0.78
Normal person, after fat meal	0.75
Normal person, after carbohydrate meal	0.81
Normal person, after mixed protein and carbohydrate	0.79
Pathologic person, carbohydrate meal	0.89
Pathologic person, after protein meal	0.89
Pathologic person, fasting	0.75

On the whole, it seems that the respiratory quotient as such can at best give only a suggestion as to the probable predominance of one material over the others in metabolism, for it is not changed by a meal composed exclusively of protein nor by muscular work.

PROTEINS AND FATS IN FEVER.

This leads to the consideration of the fate of the foodstuffs and body constituents in fever as contrasted with health and we may well begin with the protein substances, since our knowledge of the part they play is much greater than in the case of the rest. Since the work of Vogel [41] and Traube [42] it has been known that in fever the most striking change in the character of the excretion consists in the greatly increased output of nitrogen in the urine. Senator found this in his

feverish dogs and the quotation from his work just given shows fairly well his familiarity with the fact. Since these earlier papers every worker on the subject has confirmed this fact.* The amount of the excess of nitrogen varies in different cases according to the intensity of the disease and with the cause, but there is no constant parallelism between these things. Especially can it be said that the curve of the nitrogen excretion is not parallel with that of the temperature, for there are many infectious and toxic conditions in which the nitrogen output is greatly increased, but in which there is no fever at all. In the estimation of the amount of this excretion which can be regarded as abnormal, not a few difficulties are met with, as I shall try to make clear.

Speck,[43] in his review of the subject of nutritional and energy metabolism, emphasizes the idea of the existence of two kinds of protein in the body, one the living protein of the cell, which is very resistant to decomposition, while the other, the circulating protein absorbed from the food, is readily oxidized and excreted as urea. When an individual is in brisk muscular action the respiratory interchange is heightened, the excretion of urea is not much increased and the heat production and liberation of mechanical energy is thought to be due to burning of the nitrogen-free elements. It is only when these are lacking that protein is extensively used for such a purpose, and there is no store of really labile nutrition protein for this purpose. The decomposition of the organic protein is completely different from that of the unorganized and follows other laws, being inconspicuous in normal conditions, but conspicuous in certain abnormal states, such as insufficient oxygenation, various intoxications and fever. When it does break down it becomes inanimate circulating protein, but its products of decomposi-

* Leyden and Klemperer reported a loss of 100 Gm. of nitrogen equalling 3.2 kilos of muscle tissue in twelve days, and Friedrich Müller in another case determined that there was lost an amount of nitrogen (86.4 Gm.) corresponding to 2.5 kilos or 5.5 pounds of muscle tissue in eight days. Frequently, as will be pointed out, this loss is greatest during defervescence.

tion lag behind in the body and are excreted only slowly. Then, however, the urea output is incomparably greater than that appearing after muscular work and is more closely approached by that following the ingestion of excessive amounts of protein. Such disturbances are affections of the metabolism of nutrition rather than that of energy production.

It is evident from this, as I have emphasized before, that in order to obtain any unassailable information as to the character and source of the protein excreted and the amount of its excess, we must study it either in an animal in a state of complete starvation or in an animal reduced to nitrogeneous equilibrium in which we know precisely the amount of protein ingested and can estimate the respiratory changes.

That the nitrogenous excretion in fever is increased above the normal and above an amount which could be accounted for by the food is perfectly evident from the results of all experimenters. Senator estimates the amount as about double that found in the normal, but it differs in different cases and tends to sink in the later stages of the fever, always remaining above the normal, however. In those diseases in which there is a sudden or critical fall in temperature there is often an especially intense epicritical excretion of nitrogen after the crisis. This is explained sometimes as due to the retention of nitrogenous excreta in the body until that period, or again as the result of the febrile inhibition of urinary secretion. Another explanation is found in the idea that the exudate of cells produced during the inflammatory process which caused the fever is suddenly absorbed and its nitrogenous portion excreted; but this suggestion is weakened by the fact that the same epicritical rise may occur in inflammatory diseases in which there has been no such accumulation of exudate.

For an exact study of the nitrogenous excretion in fever which satisfies our demand for certainty rather than conviction we may refer to the experiments of May and Staehelin once more. May found that in his rabbit with the advance of fever and without food the nitrogenous output, which by that time must depend on destruction of the tissue proteid, advanced

from 1.79 to 2.45 Gm. per day, while Staehelin found that the negative nitrogen balance which appeared during the prodromal period of the fever increased gradually until the death of the animal from 0.5 to 4.70 Gm. per day. There is no question, then, that in this instance, in spite of the available food, there was an actual attack on the tissue proteid, and we are led to inquire whether this is always the case or whether possibly, as May maintained, the destruction of tissue protein is due to an insufficient supply of nitrogen-free and nitrogenous substance in the nutriment. The very fact that in hunger the extent of the protein decomposition is so promptly changed by the onset of fever seems almost sufficient to decide this question, but it is put in a clearer light by the experiments of Weber,[44] who studied the changes which he could produce in a sheep which he could render feverish by injection of a toxin extracted from glanders bacilli, by the administration of different amounts of carbohydrate in the food. He found first that, although it took an amount of nourishment which would under normal conditions maintain nitrogenous equilibrium and yield a sufficient amount of available energy, it still lost protein during fever. When, however, he first elevated its protein store by abundant feeding and continued the abundant feeding of protein and carbohydrate he found that even during fever there might be maintained a saving of protein. He was not able to estimate exactly the protective action of the carbohydrate or protein consumption during fever, because of the difficulty in getting the animal to consume enough of it. As F. Müller[45] points out, we can not assume from any such experiment that the protein decomposition in fever is the same in character as that in health, nor can we demonstrate in this way the existence in addition to the ordinary protein destruction, of another form due to the cause of the fever. P. A. Shaffer[46] has in the same way been able to protect the body proteid from consumption in typhoid fever by the administration of a diet rich in carbohydrates.

It is interesting to compare the nitrogenous excretion in forms of hyperthermy not produced by infection with that

found in infectious fever. Such, for example, are the hyperthermy following so-called heat puncture and that resulting from overheating. In both of these conditions it is found that there is an excessive excretion of protein as well as an increase in the general oxidation processes. This is particularly striking in the experiments made by immersing persons in a hot bath as carried out by Winternitz, in which he found that the absorption of oxygen and the excretion of carbon dioxide was far in excess of that observed in any infectious fever, and approached more closely the effect seen in violent muscular work. Others, such as Topp,[47] Schleich and Formanek,[48] have shown that the protein output is also greatly increased. On the whole, the result seems to be very similar to that which might be produced not only by muscular work, but exhaustion from such work. It would be a matter of extreme interest to determine in this case whether the protein decomposition is due to the insufficient supply of carbohydrate only, or whether it is actually the result of the heat injury to the muscles. Winternitz's experiments were carried out on fasting persons, and it seems possible that had these persons been supplied with very abundant carbohydrate food the excessive nitrogen excretion might have been spared, as, indeed, was the case in Voit's dog.[49]

We are hardly in a position to determine even from these experiments as they stand to what extent the height in temperature as such brings about an increased protein decomposition, but the general assumption is that it plays a considerable part. It is none the less a fact, supported by very abundant evidence, that when the utmost effect of hyperthermy is taken into consideration there still remains in fever an excessive excretion of nitrogenous material which must be accounted for by direct injurious action on the protein of the agent which causes the fever.

The nature of this injurious process, which is very indefinitely spoken of as a toxic destruction of the cells, must differ widely in different cases, and must in many instances be quite out of proportion to any effect which it produces in the form of fever, for it is well known that there are many

infectious and toxic processes associated with extreme destruction of the tissues in which there is no febrile reaction at all. It is, therefore, quite impossible for us to form any estimate from the composition of the excreta as to the extent of the febrile reaction which may have occurred. Nor is it possible in the present state of our knowledge for us to recognize any peculiar type of alteration of the tissue which can be regarded as particularly associated with the discharge of this reaction.

There seem to be at least two distinct types of protein decomposition which may take place in the tissues: first, that in which the protein stored in the tissues is decomposed by the action of the living cell itself, and, second, that which goes regardless of the influence of the cells, and in dead protoplasm as the result of the activity of certain ferments which are commonly spoken of as proteolytic ferments, and which play such an important part in the process of autolysis. It seems quite possible that these ferments or similar ferments may also be concerned in the vital activities of the cells, and form the instrument by which they produce the necessary decomposition of protein material, but we are quite certain of their activities in the process of autolysis.

How these processes are affected by the injurious agent in fever is not perfectly clear, but it may well be imagined that by the destruction or injury of cells, more material is furnished for the autolytic decomposition of protein which differs in some degree from the vital destruction of protein. Some light is thrown on this by the recent discovery by Aronsohn and Blumenthal,[59] that there occurs in the course of fever a very greatly increased amount of proteolytic ferment in the muscles, while that in the liver is somewhat diminished.

The biologic significance of this destruction of tissue is by no means clear, but Kraus makes the suggestion that it may prove to be explicable by hypotheses similar to those which Ehrlich adopts in his theories of immunity, and that, in fact, it is probably in some way associated with the development of immunizing or protective substances. This will be referred to later.

When we attempt to assign to a source in the body the protein which we may thus assume to be derived from living tissue, we have very little basis on which to go. Salkowski concluded from the fact that in fever an excessive excretion of potassium accompanies the increased output of nitrogen, that the nitrogen is probably derived chiefly from the muscles, but other than this we have very little information. In view of the fact that the total oxidation is not greatly elevated in fever, it is important to know whether the protein is completely oxidized in the body or whether possibly intermediate products derived from the non-nitrogenous portions of the protein are stored there. This, as is well known, was maintained by Senator, who thought, as we have mentioned before, that the body became during fever richer in fat. Fraenkel, too, finds it easier to explain the excessive excretion of urea on the basis of inadequate oxidation than otherwise, for an analogue is found in such types of intoxication as phosphorus poisoning, in which an excessive amount of urea is excreted and fat at the same time stored up. This brings us to the discussion of the question of fat metabolism in fever, which may be considered parenthetically here, since we know so little about it. It still remains a question as to the degree of oxidation of fat in the course of fever, and, as I have said, the older ideas of Senator begin to be contradicted by such work as that of Staehelin, in which an excessive consumption of the body fat can hardly be ascribed merely to inanition. Most authors writing on this subject assume that the gradual wasting of the body fat during fever is due not so much to a direct attack on the fat itself as to the lowering of nutrition and the consequent demand on fat tissues such as occurs in hunger.

Further, it is of interest to know whether the increased heat production during fever is accounted for by the oxidation of such nitrogenous substances as correspond to the increase of nitrogen excreted. It is difficult to decide this accurately, but it is generally assumed (Krehl, Kraus and others) that it is probably sufficient. After all, since the actual increase in heat production is very moderate and in some cases may be very

slight indeed, it is easy enough to accept this conclusion, but we are by no means so confident in assuming that the increased heat production does depend solely on the increased nitrogenous decomposition.

The question as to the changes in the proportions of the various nitrogenous substances in the urine brought about by fever is probably a very important one, but our information on this point is not as yet wholly satisfactory. Notwithstanding the fact that the urea output is absolutely increased, the increase in the excretion of other nitrogenous substances may be such that it actually forms a smaller percentage than normal.

Great emphasis was laid by Krehl[41] on the appearance of albumoses in the urine in fever; and there were forms of deutero-albumose which he regarded as particularly characteristic of febrile excretion. Indeed, he carried this idea so far as to assume that the formation of albumoses might furnish the real cause of the febrile reaction, and showed that the injection of such albumoses derived from various sources would cause fever in animals. He has retracted these ideas, however, since it has been shown that the fever thus produced is probably due to impurities, and since it has further been shown, especially by Dietschy,[42] that the occurrence of albumoses in the urine is by no means constant in fever, and that it depends chiefly on the reabsorption of cellular exudates and tissue remains.

Of the other nitrogenous substances the ammonia seems to play an especially important part, being excreted in greatly increased amount in some febrile diseases. Erben[43] records the excretion of 3.53 Gm. in one day in a case of measles and Hallervorden[44] and others have recorded daily excretion of 1.75 to 2 Gm., although the normal is not more than 0.7. This is doubtless due to the formation of certain organic acid materials in the course of these diseases which demand neutralization by the ammonia set free in the decomposition of the tissues.

There is at times a moderate increase in the excretion of uric acid, although this is by no means a very constant or conspicuous feature of the febrile urine, and, as Horbaczewski pointed out, it depends largely on the amount of destruction of cellular material that has taken place. Thus, we might expect in such a disease as pneumonia, and especially in the epicritical period, an increase in the uric acid output because of the dissolution of such great quantities of nuclein-holding cellular exudate, but an extensive destruction of any other tissue might be expected to bring about in the same way an increased uric acid output.

Erben points out the fact that both xanthin bodies and amino-acids are increased in fever, but in a degree varying with the char-

acter of the disease, for while these substances are increased alike in measles and diphtheria, the amino-acids are much more increased than the xanthin bases in scarlatina and typhoid fever. He explains the prominence of the amino-acids on the basis of the extensive resorption of lymphatic tissue which occurs in those diseases. The following table gives a few of the figures from his extensive investigations, which in the main agree with the earlier ones of Von Jaksch.[55]

	Percentage of nitrogen from					Total nitrogen Gm. per day	
	Ammonia	Uric acid	Xanthin bases	Urea	Amino acids		
Measles—							
Fever day ..	9.90	3.23 Gm.	1.39	4.68	77.88	6.15	29.433
Normal day .	3.70	per day	1.53	3.86	82.96	7.95	9.79
Scarlatina—							
Fever day ..	8.66		1.42	2.19	77.98	9.75	9.69
Fever day ..	10.16		2.81	3.44	71.14	12.17	3.71
Convalescence	3.78		1.47	2.33	85.86	6.56	9.07
Typhoid Fever—							
Early in fever	4.55		1.81	1.14	87.27		14.63
Defervescence	2.94		1.46	0.99	77.45	14.71	13.92

A. R. Mandel[56] has studied the excretion of xanthin bases in fevers and finds them markedly increased and inversely proportional to the output of uric acid. He further shows that there is a constant relationship between the height of the fever and the quantity of purin bases in the urine and that a febrile temperature can be produced by the injection of xanthin bases. It seems from this work that we have not reached a position in which we can finally estimate the importance of the metabolism of these substances, but it is probable that their further study will throw much light on the significance of the intensified decomposition and excretion.

Much importance has recently been attached by Folin[57] and others to the excretion of kreatinin in its relation to the nitrogenous metabolism as a whole, and it seemed probable that in such conditions as fever in which there is a great wasting of muscle and other tissues there might be expected an increase in its excretion.

Leathes[58] in his observations on febrile patients in the wards found an actual decrease in the amount of this substance as compared with the normal, but later having kept himself on a kreatinin-free diet he experimented on himself, producing fever by injecting an antityphoid vaccine and then found a definite although slight increase in the amount of kreatinin excreted. Shaffer,[59] on the other hand, does not regard kreatinin as a constant index of endogenous protein katabolism. He finds it slightly increased in acute fevers regardless of the muscular development of the individual. Kreatin also is found by him to be excreted by the subjects of acute fevers, and he thinks that it results from an abnormal breaking down of muscle protein. Van Hoogenhuyze and Verploegh[60] conclude that kreatin may be converted into kreatinin chiefly by the liver and that when the liver is exhausted or injured so that its ability to transform the

kreatin into kreatinin is disturbed, kreatin may also appear in the urine. In several cases they found kreatinin markedly increased during high fever. In the case of one of themselves this was especially clearly demonstrated, as a brief febrile attack occurred during their study of their own kreatinin excretion. Their result differed from that of Leathes in being associated with no great increase in the general nitrogen output.

In addition to the above-mentioned substances, there frequently occur in febrile urine one or more of the stages in the oxidation of oxybutyric acid into diacetic acid and acetone. The acetone is that stage which is most likely to appear, and it is only in more intense disturbances of oxidation that the diacetic acid and oxybutyric in turn appear. In convalescence they disappear in a reverse order. These substances are doubtless formed in some such way as they appear in inanition, but practically never reach the abundance in which they are present in that condition. They may be made to disappear in part, at least in some cases, by the administration of carbohydrates. They are not present in fever to any such extent as observed in diabetes, but when a febrile affection supervenes in diabetes the acetone bodies are usually increased. As to the source of these subtances, it seems quite probable that they are directly derived from the decomposition of protein material, whether from the inanition which so commonly accompanies fever or from the toxic effects of the infectious process, but they may be an index of the febrile abnormal consumption of fat.

Regnard,[61] Geppert,[62] Minkowski,[63] Kraus [64] and others have shown that in fevers the carbon dioxide content of the blood is diminished and think that this is due also to an acid poisoning which, however, must be somewhat indirectly demonstrated. The increased output of ammonia mentioned above is another indication of the same thing.

CARBOHYDRATES IN FEVER.

With regard to the oxidation of carbohydrates in fever we know very little. There are numerous statements in the literature concerning the disappearance of glycosuria in diabetes when fever supervenes.

Bleiweis [66] showed that this was inconstant and that indeed the assimilation limit for sugar might be lowered in febrile diseases. Richter [68] confirms this inconstancy of the effect of fever on glycosuria and diabetes and also makes the statement that in fever, alimentary glycosuria is easily obtained. It can be shown that it is the infection and not the elevation of temperature which stops the glycosuria in diabetes, for the hyperthermy resulting from heat puncture has no effect on adrenalin glycosuria, while bacterial infection with fever prevents such glycosuria.

The whole subject of alimentary glycosuria in fever has been considered by Di Campagnolle [67] who finds that the limit of assimilation of dextrose is markedly lowered. Ott [68] found that in febrile animals the storing and subsequent conversion of glycogen occupied a much shorter time than in normal animals, and he suggests that in man the exhaustion of the stored glycogen may occur more rapidly than in health. Noel Paton [69] also finds from experiments on rabbits that the simple elevation of temperature increases the rapidity with which the glycogen of the liver is converted into glucose, and that in infective fevers the glycogen in the liver is quickly reduced. An interesting paper is that of Hollinger,[70] who studied the sugar content of the blood in health and in fever. The normal content is greatly exceeded in almost all cases of pneumonia which he studied. To such an extent did this hyperglycæmia rise that had these cases been diabetics there would certainly have been glycosuria, but none occurred here. In other forms of infection also he finds a similar hyperglycæmia.

May and Weber, as we have already mentioned, held to the idea that they could by the administration of carbohydrate during fever reduce protein destruction to, or even below, the normal amount, and, indeed, they were able to reduce the febrile destruction of the body protein to a very great degree. The exact explanation of this is not perfectly clear even yet; whether they merely supplied the deficiency resulting from inanition which would otherwise be supplied by protein, or protected protein from the effect of the hyperthermy alone, or whether finally the carbohydrate given could protect to some extent the protein of the tissues from toxic destruction (which

seems entirely improbable) it is difficult to decide. Weber, however, admitted that he was unable in this way to protect the protein completely.

Hirsch, Müller and Rolly, in their studies of topography of heat production, have emphasized the importance of the liver in this process because they found that it was the warmest organ. Hirsch and Rolly [25] investigated the effects of heat puncture on a curarized animal and found that fever could be produced in these animals, which lends support to the idea that the heat production occurs in the liver, and that it depends on the non-nitrogenous metabolism of the liver. It was but a short step to the investigation of the effect of heat puncture in a glycogen-free animal, and Rolly [71] found that in such an animal heat puncture is impotent to produce any elevation of temperature. If, however, the glycogen be restored to the animal, fever production takes place. Infections, however, give rise to fever in glycogen-free animals quite as well as in those with abundant carbohydrate supply. Senator and Richter [72] repeated this work and found that after making the animal glycogen-free by poisoning with strychnine the heat puncture produced almost as high a temperature as in well-nourished rabbits, and they conclude that the production of fever is not dependent on the presence of glycogen, and, indeed, that hyperthermy does not depend on the burning of any special substance. In my judgment, the positive results of Senator and Richter must have greater weight than the negative results of Hirsch and Rolly, especially in view of the uncertainty which attends the somewhat difficult operation of heat puncture in the production of fever, and we are thus deprived of the brilliant explanation of heat production which Hirsch, Müller and Rolly attempted to furnish us. It seems evident, however, from the other experiments and observations, that carbohydrates are well oxidized in fever, and perhaps even particularly well if we accept the results of Hollinger. At any rate, it appears that in the fevers that have been studied the carbohydrates are rapidly oxidized and may even protect to a certain degree the proteins which are otherwise so quickly attacked, and we may

the more readily assume that in the later stages of fever in which no great quantity of carbohydrates has been given in the food the oxidation finally depends chiefly on the protein and fat.

WATER METABOLISM IN FEVER.

As to the water exchange we again have rather conflicting ideas which doubtless depend on inaccuracy of observation and on complications which are not necessarily found in all febrile affections. Such complications may arise, as is obvious, from disease of the kidneys or of the heart or from general disturbances of the mineral metabolism which depend on degenerative processes in the tissues as a whole. Here, again, it is the water balance which must be accurately determined so that the observations of Glax,[73] who neglected the respiration, perspiration, etc., and decided on water retention in fever, can hardly be considered. Somewhat more exact are the investigations of Riva Rocci and Cavallero,[74] who weighed their patients and estimated the respiratory exchange, and thus calculated the water excretion from the skin and lungs. They found a variable retention during fever, but a great output of water at the end of fever. Krehl and Matthes found that the heat loss in fever from evaporation preserved its relation to the heat loss by radiation and conduction—an abnormal condition—because in health the heat loss by evaporation is extraordinarily variable and after muscular exertion the increase is enormous in comparison to that by radiation and conduction. Wassilewsky [75] finds in the study of many febrile diseases that the evaporation from the skin is very low during the stage of increment of the fever, somewhat higher but still below the normal during fastigium, but greatly increased in the stage of decrement. Lang [76] experimented with tuberculin fever and found that there was a marked difference in fever between the evaporation of water by the lungs and by the skin, for, while in health the excretion by the skin per square metre may be 13 Gm. per hour, increasing 100 per cent. after an abundant meal and very much more after muscular work, it is also about 13 Gm. during the height of fever. On the other hand, the normal man

exhales 0.21 Gm. per kilo from the lungs and 0.27 after a meal. The febrile patient may exhale while fasting 0.32 Gm. per kilo from the lungs. Thus in fever the evaporation from the lungs is increased 50 per cent. and the total loss of water is 20 per cent. Thus the insufficiency of the evaporation from the skin in fever must have a very important rôle in the elevation of the temperature. It must be suggested, however, that in this work of Lang only one type of fever is considered, whereas it is well known that in certain other types, such as that seen in tuberculosis and acute rheumatism, there may be a great deal of sweating associated with high fever.

Schwenkenbecher [77] has devoted much attention to the water exchange in fever and finds that the excretion of water varies perhaps more with the "direction" of fever, as he expresses it, than the actual height of temperature. He finds that in pneumonia there is, on the whole, no marked retention of water, although that is usual in typhoid fever and tuberculosis in the sense that, although the patient loses more water than the healthy person, he also excretes more solid substance and may become relatively water rich. He follows Liebermeister in his ideas of heat regulation, finding that the febrile person with his elevated temperature excretes water when the heat production is still further elevated by a meal, in the same proportion as the normal person. The amount of water at the command of these mechanisms is very considerable, so that until we have more accurate knowledge of the water balance we can not certainly assume that the diuresis which accompanies the crisis in certain fevers is dependent on a previous active retention of water. It is clear that these observations of Schwenkenbecher and Lang and others give us information only as to the alterations of the water exchange which have to do with heat regulation, while the total water metabolism is not elucidated by this means. For that purpose we must have such absolute figures as are to be found in Staehelin's table, from which we learn that the dog infected with surra took in during the whole period with the food and drink 9030 Gm. of water and excreted altogether 11,225 Gm., the water of urine, fæces and respiration

being measured. The dog thus lost during this period 2195 Gm. of water, which, in spite of the fact that some œdema developed, does not argue for the retention of water.* On the whole, while the diminution in the urinary output during the height of fever and the concentration of the urine are well known in contrast to the increased urinary output at the crisis or in the early days of convalescence, the attempts made to demonstrate, in consequence of this, increased water content of the blood and tissues have not been distinctly successful or convincing. The explanation of the decrease in the amount of urine has generally been attempted on the basis of disturbances in the blood-pressure and pathologic changes in the kidneys interfering with their function. It seems, however, that in view of the sudden restitution of the functional activity of the kidneys in the crisis, the disturbance must have been a functional one rather than an actual anatomic lesion, and one can hardly escape the idea that if water is retained in the body during fever it is not because of the inability of the kidneys to excrete it, but rather because it is needed in the tissues to serve some good end in the furthering of the febrile reaction. Much more accurate work is needed for the clearing up of this subject.

MINERAL METABOLISM IN FEVER.

Of the metabolism of mineral salts in fever we can speak with certainty only of the relation of the sodium chloride. A distinct retention of the sodium chloride in the tissues seems to occur in certain febrile diseases, although not in all. Redtenbacher [79] was the first to point out such chloride retention in pneumonia, and his discovery has been abundantly confirmed by Röhmann,[80] Terray,[81] Moraczewski,[82] Hutchinson [83] and many others. It is found that the amount of sodium chloride excreted in the urine in pneumonia is very greatly decreased

* In another paper [78] Staehelin intimates the effect of night sweats in tuberculous patients and finds that sweat as such has no influence on the consumption of energy and has not nearly the cooling effect that might be expected, and that its significance, therefore, must be sought elsewhere than in the heat regulation.

until the crisis, when it is excreted in large amounts. This is true even though an increased amount of salt be given in the food. Röhmann thought that the retention was due to a combination of sodium chloride with circulating proteins in the blood, but this has been disproven, and it is shown that the accumulation really takes place in the tissues. Leyden, who emphasizes the retention of water during fever, held the view that the retention of chlorides was for the purpose of maintaining the isotonicity of the tissue fluids, and in this Terray agreed, but it seems, as Hutchinson points out, that the amount retained is far greater than is necessary for that purpose, and some other explanation must be found. This is the more difficult, since other fevers, such as typhoid, show no such constant chloride retention. Another suggestion is that the chlorides actually forming a constituent of the excessive exudation in pneumonia may explain the retention, but even this, as Terray has shown, is quite insufficient to account for the amount retained. In malaria it has been found by Rem Picca and Caccini[84] and also by Terray that the chloride retention is to be observed on the fever-free days, while there is an increased output on the days of fever, and they think that the great destruction of red corpuscles is sufficient to account for this—again an improbability.

We know so little about the excretion of other mineral substances in fever that we can hardly resort to the consideration of the balance usually maintained between these substances in order to explain the divergence from normal in the relation of the chlorides. It may have a direct relation to the excretion of phosphates, as Schwartz thought. There is a great deal of dispute about phosphorus excretion in fever, but Moraczewski tells us that the phosphorus excretion is increased, especially with respect to the acid phosphates. The relation between the phosphorus excretion and that of the calcium is well known to be intimate, and it is stated that the calcium excretion is diminished in fever, but that after convalescence this retention of calcium phosphate is compensated for by an increased excretion. Perhaps the most satisfactory paper on the subject is the recent one by Moraczewski,[85] in which cases of pneumonia, typhoid, malaria and tuberculosis are studied. All the assertions of the earlier writers are confirmed as far as they go, and in pneumonia a very distinct critical rise in the phosphorus and potassium excretion is noted, the excretion of these substances re-

maining high after the crisis, while the chlorides are extremely low until several days after the crisis, when they suddenly increase. Moraczewski gives a scheme to illustrate the relations of chlorides and phosphorus to the temperature curve, which he divides into several periods. The onset of the fever brings with it an increase of chlorides, decrease of nitrogen and decrease of phosphorus. In the second period the chlorides decrease, nitrogen increases and phosphorus decreases, but begins to rise a little later with the nitrogen. From this condition, in which the chlorides fall very low, there occurs a change with the fall of temperature which consists in a continued increased output of nitrogen and of phosphorus, while the chlorides increase to normal or above it, all finally returning to normal.

Let us now, after this somewhat wearisome discussion of the details of the metabolism in fever, review very briefly what has been said about the whole subject. We find that in fever we have a reaction which is occasioned by a variety of injurious influences acting on the body, a reaction which is characterized by a moderate increase in the oxidative processes, and consequent moderate increase in heat production, but more especially characterized by such changes in the heat regulation as lead to disproportion between the heat production and the heat loss, and a consequent rise in body temperature. The disturbances in metabolism are found to consist, not especially in the oxidation of non-nitrogenous substances which are predominately concerned in health in the production of heat, but in much greater degree in the destruction of protein material, not the protein of the food exclusively, but in a pre-eminent degree the protein of the tissues. The destruction and oxidation of carbohydrate and fat goes on in fever also, possibly to a greater extent than has generally been supposed. The changes in mineral metabolism are as yet but imperfectly understood, but it appears that in the course of fever, water and certain mineral salts are at times retained in the body only to be excreted later when the febrile process is over. On the whole, the change in metabolism is not one particularly marked by its intensity, but rather by the peculiar proportions in which the materials are concerned, and the formerly accepted idea that there we have to deal with a greatly increased oxidation is upheld neither

by the amount of heat production nor by the quantity of oxidation products resulting. It seems from the constancy of the characters of the febrile reaction, no matter what the exciting cause of the fever be, that we can not regard it as the mere effect of the injurious agent on the passive body, but must rather look on it in the light of an elaborate modification of chemical processes evolved in the course of centuries of development to answer some special purpose. It seems probable that every detail of this reaction is that which is best calculated to take its own special part in the making up of a whole well-aimed plan. Every available mechanism co-operates to the uttermost of its power in the first stage to elevate the temperature of the body; when that temperature is once brought to a suitable level, many of these mechanisms are no longer necessary, and when the febrile temperature itself is no longer needed other mechanisms are equally active in discharging the heat. The aim of the individual peculiarities of metabolism is not so easily grasped, but it is here again quite as difficult to escape from the conviction that they are designed to play their part in the general plan, and because this reaction has developed in the long processes of evolution, it seems inevitable that this plan is one devised for the good of the organism, and that fever is in its essentials a protective reaction. This idea is not by any means new, but has prevailed for centuries, being lost throughout whole periods in the struggles of physicians to cure the fever regardless of the disease, and it is only in recent years that it has again become usual to think of the fever as probably a beneficial reaction which should not be interfered with.

Some light has been shed on this by the controversies regarding the usefulness of antipyretics, about which a very considerable literature has sprung up. In just what way the fever acts beneficially it is almost impossible to say in the present state of our knowledge, but the recent studies of the development of immunity, which show that when the tissues are attacked and injured they react by developing an immunizing substance, probably offer a general explanation for the type of

reaction with which we are dealing, which agrees in so many particulars with the behavior of the organism in the known development of immunity. The very facts that when the injury is overwhelming we may have no febrile reaction, and that when the course of the intoxication suddenly changes to the advantage of the organism the fever ceases, point to a close analogy between the febrile process and the process of the production of immunity. Why the heightening of the temperature of the body should be necessary is not perfectly evident, but it is easy to believe that under those circumstances the production of immune substances might proceed in a way impossible at a lower temperature. In conclusion, then, I can only express my conviction that the febrile process is a reaction beneficial to the organism and doubtless intimately associated with the development of protective substances to combat the injurious agencies which have invaded the body.

NOTE.—Since the delivery of this lecture there has appeared the very interesting paper of Rolly and Meltzer (Deutsch. Arch. f. klin. Med., 1908, xciv, 335) on the significance of hyperthermy. They find the growth of bacteria somewhat inhibited by temperatures of 40–41 C. No certain result could be obtained as to the protective effect of overheating on animals infected with bacteria when large quantities are injected at once. If, however, smaller quantities are injected daily there is a distinctly favorable influence. Heating alone does not change the alexin content of the blood, and the effect of heating on phagocytosis is indefinite in the animal, but up to 40 C. is apparently favorable *in vitro*. Heating has no influence on the resistance of animals to fatal doses of a bacterial poison, but when small doses are given agglutinins and bacteriolytic substances are produced far more rapidly and abundantly in animals which are kept overheated than in those which are kept cool.

The febrile temperature injures the organism to a certain extent by causing loss of weight, lowering the hæmoglobin index, etc., but there are no definite injuries produced in the organs even by a long heating. Even though overheating can not be regarded as surely identical with the condition in fever, it is probable that this paper marks a most important advance in the comprehension of the true nature of fever.

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METABOLISM IN DIABETES*

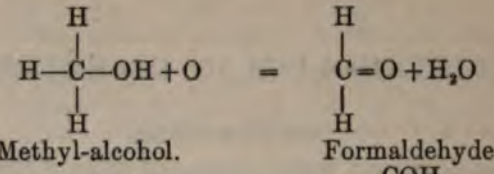
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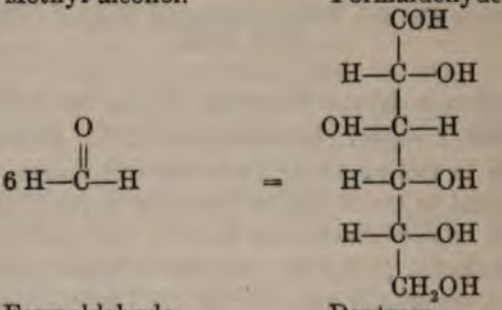
IN my student days in Germany during the winter semester of 1888, I visited the laboratory of the botanical garden of Munich to call on Oscar Loew, who had some time before been assistant to Dr. R. Ogden Doremus in the City College of New York. Loew showed me how he was passing the vapor of methyl-alcohol (CH_3OH) over hot oxidized wire gauze and collecting a product in water which was formaldehyde (CH_2O). This he shook with milk of lime and obtained, after further treatment, a syrup which was as sweet as sugar and which represented the condensation of six molecules of formaldehyde into one of sugar. This artificially prepared syrup gave many reactions for sugar, but did not rotate polarized light nor was Loew able to crystallize it. At the time I had been preparing levulose in Voit's Munich laboratory. I told Loew of the difficulties of the crystallization of levulose, and that my levulose syrup would crystallize only when crystals themselves were added, and at his request I sent him some of mine. But, alas, the effort was futile. I can never forget the alternate enthusiasm and despair with which he tried to discover the hidden secret of his precious syrup. About this same time Emil Fischer showed that the substance which Loew had in his hands consisted of that kind of levulose which is optically inactive, being a mixture of right and left levulose, and Fischer indicated how levulose could also be transformed into dextrose.

* Lecture delivered November 21, 1908.

The changes described are according to the following formulas:



Methyl-alcohol. Formaldehyde.



Formaldehyde. Dextrose.

The triumphs of pure chemistry are leading nearer and nearer to a more perfect critique of the processes underlying biologic phenomena. Long ago Baeyer suggested that the formation of sugar in the leaf was through a condensation of formaldehyde molecules into dextrose and this year Grube [1] has shown that formaldehyde perfused through the liver of a tortoise is converted into glycogen.

Thus the laboratory may throw a vivid light on questions of fundamental significance in the biologic world. It is from the laboratory standpoint that I wish to direct attention to the diseased condition known as diabetes. Some may question the right of a laboratory man, a physiologist, to present to medical men a scientific discussion of a diseased condition. In defense I can only quote to you the stirring words of Magendie written in Paris as long ago as 1836 as an introductory to his "Elements of Physiology," a copy of which I inherited from my father's library. Magendie said: "In a few years physiology, which is already allied with the physical sciences, will not be able to advance one particle without their aid. Physiology will acquire

the same rigor of method, the same precision of language and the same exactitude of result as characterize the physical sciences. Medicine, which is nothing more than the physiology of the sick man, will not delay to follow in the same direction and to reach the same dignity. Then all those false interpretations which, as food for the weakest minds, have so long disfigured medicine, will disappear."

Let us, then, inquire into the pathologic physiology of the man sick with diabetes.

Diabetes mellitus is a condition in which the power to burn sugar within the organism is partly or completely destroyed. This condition is not to be confounded with that of glycosuria, which occurs when the sugar-holding capacity of certain organs has been reduced or overstrained.

Claude Bernard's celebrated experiment, called *la piqure*, in which he pricked certain nerve-cells lying in the floor of the fourth ventricle, resulted in the appearance of sugar in the urine. Bernard named this group of cells the "diabetic centre," and from this experiment has arisen the erroneous belief that diabetes is essentially of nervous origin. It has, however, been clearly demonstrated by Dock [3] that *la piqure* does not cause sugar excretion if the animal experimented on be fasting; that is, if the organs be free from glycogen. It is, therefore, apparent that the nerve impulses from the so-called diabetic centre simply reduce the capacity for holding glycogen on the part of the liver and perhaps of other organs, with the result that the blood is flooded with sugar which is eliminated by the kidney.

Macleod and Dolley [3] show that after injection of nicotine, which acts to prevent the transmission of impulses through sympathetic ganglia, stimulation of the diabetic centre causes neither glycosuria nor loss of liver glycogen.

The "starvation diabetes" of Hofmeister [4] is another example of glycosuria in which sugar ingested readily appears in the urine, because of a weakened power of the organism to retain it as glycogen. Even in normal health unlimited quantities of sugar can not be given without a portion appear-

ing in the urine. Thus, Moritz [5] found 2 Gm. of dextrose in the urine of a normal man who had received 200 Gm. in his food. Here the dose evidently surpassed the regulatory capacity of the glycogenic function. Moritz [6] also found sugar in the urine in the cases of four out of six men who had liberally partaken of carbohydrate food and champagne.

Another type of glycosuria, discovered by Von Mering,[7] occurs after the administration of phlorhizin.* There the quantity of blood sugar is reduced, since the blood coursing through the kidney has no power to retain its sugar. Hédon,[8] in one phlorhizined dog, found sugar in the blood in an amount too small to determine at a time when the urine contained 11 per cent. of dextrose. Small quantities of sugar given in phlorhizin glycosuria are completely eliminated in the urine,[9] but if large quantities be ingested the organism is found fully able to burn sugar. Prolonged fasting does not entirely remove all the glycogen from a phlorhizinized dog,[10] but cold or mechanical work is able to do so.[11]

Von Mering and Minkowski [12] removed the pancreas from dogs and obtained a condition which was markedly analogous to diabetes mellitus in man. There is hyperglycæmia and a large excretion of dextrose in the urine; ingested dextrose can not be burned, but is completely eliminated. The dogs show a considerable acidosis, with excretion of beta-oxybutyric acid, and they die in coma.[13] If a portion of the gland remain in the abdominal cavity there is either no diabetes or only a partial diabetes. If a portion of a pancreas be transplanted into the abdominal cavity of a depancreatized dog, the diabetes is stopped or reduced as long as the transplanted piece remains functional. Such experiments as have been made in man have not been successful. Minkowski [14] reports that if a piece of the pancreas be ingrafted under the skin of a dog and afterward the whole of the remainder of the pancreas be removed from the abdomen, the dog's urine remains free from sugar

* This spelling of the word was determined by Von Mering and should be universally adopted. Lépine writes, "En tout cas, au point de vue de l'étymologie, phloridzine est un barbarisme."

for two months, but on extirpation of the piece ingrafted under the skin an extreme diabetes sets in.

Minkowski [15] early noticed that the livers of depancreatized dogs were free from glycogen. This is further emphasized by the recent experiments of Allard,[16] in Minkowski's laboratory, who has shown that severe cold will not increase the sugar output in completely depancreatized dogs. The effect of cold is to produce shivering which would convert into dextrose any available glycogen, were such within the organism.

Curiously enough, although the depancreatized dog is free from glycogen, and ingested dextrose can not be converted into glycogen, yet when levulose is given glycogen may be largely stored in the liver. The capacity for glycogen formation is, therefore, intact. It would seem that when the cells of the organism were hungry for dextrose then an inhibition was laid on the liver, preventing its storage of glycogen from dextrose. Bang [17] finds no glycogen in the livers of depancreatized dogs, but finds that these same livers contain a diastatic ferment which acts energetically on a solution of glycogen. Bang and his pupils [18] have shown the same thing to be true in phlorhizin glycosuria.

Zuntz [19] removed the glycogen from a normal fasting rabbit by strychnine convulsions, and after 119 hours of further fasting found 1.3 Gm. of glycogen in the liver and muscles. Hence the normal fasting organism has the power to construct glycogen. But in both pancreas diabetes and in phlorhizin glycosuria [20] this power to convert dextrose into glycogen is absolutely lost.

From this discussion it is evident that when dextrose can not be burned in the organism the synthesis of glycogen from dextrose is in abeyance, whereas the reverse effect, the conversion of glycogen into sugar, is entirely normal.

The facts already noticed lead to the important conclusion that exposure to cold, which brings about an adaptive increase in heat production by greatly increasing the fat combustion, does not increase the sugar output in either pancreas or phlorhizin diabetes. Hence the sugar output is not connected with

the quantity of fat metabolized. It has also been shown [20] that mechanical work, to accomplish which a doubled metabolism of fat would have been required, is without effect on the output of sugar in a fasting phlorhizinized dog. Seo [21] has made similar experiments in pancreas diabetes. When there is a partial extirpation of the gland, and, therefore, only a partial diabetes, exercise reduces the sugar output; the conditions for its oxidation are improved. But when the pancreas is completely removed, then the sugar output actually increases during the working period, to be followed by a compensatory reduction, so that in the aggregate mechanical work is entirely without influence on the sugar excretion.

From this discussion it may be safely stated that the factor of fat metabolism as increased by cold and mechanical work is without influence on the output of sugar.

The glycerin component of fat when ingested alone in diabetes is convertible into dextrose (Cremer, Lüthje). It may be that when large amounts of fat are ingested the glycerin radicle may be absorbed before the fatty acid radicle, and in that way involve a small production of sugar from fat. However, A. R. Mandel and I [22] have given a phlorhizinized dog 100 Gm. of fat on a day when the dog actually burned only 69.5 Gm. of fat, and yet there was no increase of urinary sugar. We have also [23] given a diabetic man 222 Gm. of fat without affecting his output of sugar.

In the acute form of diabetes mellitus in man, there is complete loss of power to burn dextrose, and one may infer from the similarity of the conditions to those of pancreas diabetes that the tissues do not retain glycogen. It is evident that such an organism must exist at the expense of protein and fat. Within the cells of the living body, certain motions are maintained, which are manifest in such physical forms as heat, work and electricity. These material forces are not generated from nothing, but from an exact equivalent of potential energy resident in the materials burned in the body. The requirement of energy for the maintenance of the life of a man is fixed and definite and in general amounts to 32 large calories per kilo-

gramme of body substance in starvation and to 35 calories per kilogramme when an average mixed diet is taken. The diabetic who can not burn dextrose is thrown on protein and fat as sources of his potential energy. Were this an uncomplicated situation a diabetic could doubtless imitate the habits of the Esquimo, who lives on oil and meat. But it happens unfortunately that a major portion of the ingested protein is convertible into sugar in the diabetic organism, and that this sugar which is carried away by the urine may contain by far the greater part of the potential energy of protein which is available for cell life. To compensate for this, the protein metabolism increases, but fat metabolism remains the mainstay of the life of the diabetic as it does in the fasting individual. In diabetes the protein metabolism is abnormal, and conditions varying in severity also arise in which the end-products of fat metabolism, such as beta-oxybutyric acid, aceto-acetic acid and acetone, do not burn, but accumulate within the organism and are eliminated in the urine.

The sugar production from protein may first be considered and later the origin of the so-called "acetone bodies." In this discussion the organism must be considered as a chemical factory working on definite materials.

Minkowski [24] found that, after he had extirpated the pancreas in dogs, whether they were fasting or on a meat diet, there was a constant elimination of nitrogen and dextrose in the urine and that these two substances were always in exactly the same proportion day after day. There were 2.8 Gm. of dextrose for each gramme of nitrogen. These two constituents rose and fell together, but their relationship, called the D : N ratio, remained constant at 2.8 to 1. Since each gramme of nitrogen in the urine represents a destruction of 6.25 Gm. of protein in the body, it is evident that 2.8 Gm. of dextrose may arise from 6.25 Gm. of protein, or protein yields 45 per cent. of dextrose.

Cremer [25] found that, after the frequent injection of phlorhizin, in the case of one fasting rabbit, the second day's urine gave a D : N ration of 2.8, and he wondered if it were always so. This was repeatedly confirmed in my laboratory,[26] where it has

also been shown that the same ratio may be obtained in phlorhizinized cats [27] and goats.[28]

The D : N ratio of 2.8 to 1, which indicated a production of 45 per cent. of sugar from protein, apparently represented the upper limit of sugar production from protein in diabetes. Minkowski's classical work did not include an experiment in which gelatin was ingested. I, therefore, tried to determine the amount of sugar which phlorhizinized rabbits would produce when gelatin was given. It became apparent that rabbits were not satisfactory animals for this kind of diet and so I began work on a dog.[29] During the first days the known 2.8 ratio could not be obtained, and it was fancied that this was on account of a rich sugar and glycogen supply in the dog. A continuation of the experiment on another dog showed that, even on the fifteenth day of diabetes, the 2.8 ratio appeared to be as far off as ever and that a higher ratio was constantly obtained. This higher D : N ratio was 3.65 to 1 and represented a sugar production from protein of nearly 60 per cent. Gelatin was shown to yield sugar in equal amount.

The significance of this ratio was enhanced by the discovery by Mandel and myself,[23] of its existence in human diabetes when the patient was placed on a diet consisting of fat and protein. Other similar cases are now on record, one of which will be discussed later.

A remarkable discovery of the present year is that of Falta,[29] who has shown that a D : N ratio of 3.6 to 1 may exist in dogs after removal of both pancreas and thyroid.

The cause of the variability of the ratios in different animals and in the same animal under different circumstances can not be definitely given. Falta believes that the extirpation of the thyroid and pancreas leaves the adrenal in a highly active condition, furnishing a secretion which tends to promote the formation of sugar. I do not feel that this is the place for a detailed discussion of Dr. Falta's theories regarding the interaction between the pancreas, thyroid and adrenal glands as connected with the sugar metabolism, because I have already expressed my inability to accept his views. My own views are based on the

following reasoning. Loewi [30] has explained that the fact that blood sugar is not normally eliminated in the kidney is due to its chemical union with an unknown colloid. In diabetes mellitus sugar rises in the blood above the power of the colloid to unite with it and the crystalloid sugar as found within the organism is readily eliminated by the kidney. In phlorhizin-diabetes, however, the secreting kidney tubules have the power to break up the colloid-dextrose combination with the reduction of blood sugar which is eliminated in the urine. Stiles and I[31] accepted this theory and believed that the dextrose in the colloid-dextrose radicle was not combustible, because the subcutaneous injection of 5 Gm. of dextrose in a phlorhizinized dog resulted in its complete elimination in the urine. It must have been spared from combustion by its union with colloid. Mandel and I [28] have explained the cause of the difference between the 3.65 and 2.8 ratios by assuming the existence of two different chemical combinations, an alpha-colloid dextrose and a beta-colloid dextrose. By alpha dextrose is meant the sugar represented in the 2.8 ratio or 45 per cent. of the protein molecule. The beta-dextrose represents the additional 13.6 per cent. of the protein when the 3.65 ratio is present. The ratio would then depend on the combustion or non-combustion of the beta-colloid dextrose.

In my book on nutrition [33] I have called attention to the fact that in both the carbon monoxide diabetes investigated by Straub and the ether glycosuria as described by Seelig, the urinary sugar is derived exclusively from protein and may even disappear if the animal be given carbohydrates alone. This indicates the formation in protein metabolism of a distinctive chemical compound which yields dextrose. If the ether is administered with oxygen the glycosuria does not ensue. So the glycosuria is due to lack of oxygen acting on the hypothetical colloid-dextrose derived from protein. It may be that adrenalin glycosuria is of similar nature. It is certain that injections of adrenalin greatly reduce the blood supply to the tissues. Underhill and Closson [34] found in one experiment that the quantity of dextrose in the urine after injecting adrenalin in starvation was only slightly increased if the dog had first

received 7 Gm. of dextrose per kilogramme. In one imperfectly described experiment Underhill states that adrenalin glycosuria is not to be prevented by free access of oxygen. Underhill believes that adrenalin stimulates sympathetic nerves causing a discharge of sugar from the glycogen repositories of the body which bring about a hyperglycæmia. It seems to me, however, that to this may be rightly added a separation of dextrose from colloid-dextrose through the anæmic condition of the tissues.

One can explain Falta's higher ratio after extirpation of both thyroid and pancreas in the dog by assuming conditions which cause the cleavage of beta-colloid dextrose.

That a molecule of protein can yield sugar to the extent of 60 per cent. of itself seems, indeed, marvellous. It is in accord with the early idea of Voit, that protein breaks up into a nitrogenous portion convertible into urea and a non-nitrogenous portion which as sugar or fat can be used by the organism. Even as late as 1902 Rubner believed in such a simple cleavage of protein which yielded large quantities of sugar, and my own papers of the same date maintained the same view.

Kossel,[34] however, at the International Physiological Congress, held in Cambridge, England, in 1898, first drew attention to the fact that many cleavage products of protein, such as leucin, lysin and arginin, contained six carbon atoms or the same number as dextrose, and he compared an aggregation of such amino-acids forming protein with the analogous polysaccharides. On the railway train to London after the congress, Kossel explained to me that he believed these amino-acids were convertible into dextrose and that they formed the source of urinary sugar in diabetes. The same idea was later voiced by Friedrich Müller,[35] who stated that protein which yielded so large a quantity of amino-acid radicles could scarcely contain a sugar radicle equal to 60 per cent. The great work of Emil Fischer has taught that the essential composition of protein is a structure formed of chains of amino-acids. He has recently hung together eighteen of these radicles in an octodecapeptide

containing four leucin and fourteen glycocoll molecules and being l-leucyl-triglycl-l-leucyl-triglycl-l-leucyl-octoglycyl-glycin.

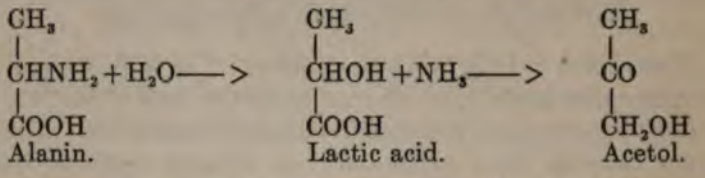
$$H_2NCH(C_4H_9)CO.[NHCH_2CO]_3.NHCH(C_4H_9)CO.[HNCH_2CO]_3.-HNCH(C_4H_9)CO.[HNCH_2CO]_3.HNCH_2COOH.$$

This forms a body akin to peptone. The high molecular complex called protein, which constitutes the basis of our being, is, after all, separable into simple chemical compounds. In the larger molecule these amino-acids are chained together, even as in structural framework various iron beams are riveted together. Digestive proteolysis or internal metabolism rends the higher structure of the molecule and leaves its individual supports, the amino-acids, open for further disintegration.

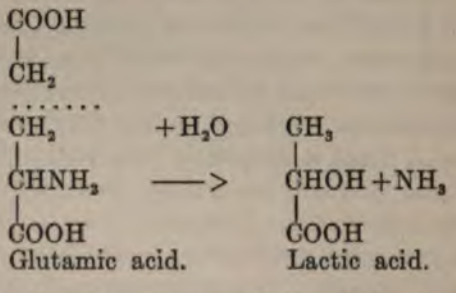
Kossel, Friedrich Müller and Hans Meyer were together at Marburg, and Knopf,[38] at Meyer's suggestion, gave an amino-amid called asparagin, commonly found in protein, to a dog which was partly under the influence of phlorhizin, and found a considerable increase of sugar in the urine. At the same time Stiles and I [37] fed a completely phlorhizinized dog with a pancreatic digest of meat, which had been carried so far as to contain only amino-acids. The result was a large production (40 per cent.) of sugar from the amino-acids ingested.

Embden and Salomon [38] have given asparagin, glycocoll, and alanin to partly depancreatized dogs and have noted large increases in the amount of urinary sugar. These experiments are wanting in completeness in that the pancreas diabetes was not a total diabetes, and the same criticism is justified concerning similar experiments on phlorhizinized dogs by Baer and Blum [39] and Glaessner and Pick,[40] which have been awarded a recognition out of proportion to their worth. The true situation was first appreciated by Neuberg,[41] who found glycogen in the liver and lactic acid in the urine of a normal rabbit following the ingestion of alanin. The amino-acid alanin is converted into lactic acid by hydrolysis with elimination of ammonia. The ammonia is converted into urea. Arthur Mandel and I [42] have shown that d-lactic acid is completely converted

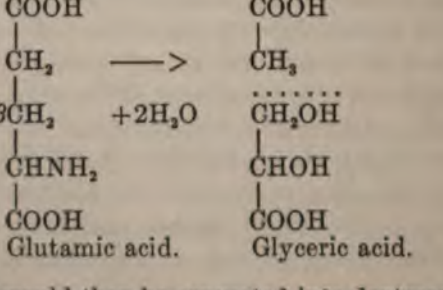
into dextrose in the organism, and recently Ringer and I [43] have given 20 Gm. of i-alanin to a diabetic dog and witnessed its complete elimination in the form of urinary sugar.



In the experiments published last June, I showed [44] the probability that glutamic acid was convertible into sugar in so far as it could form alanin in the organism. This would take place according to the following reaction:



Or it may be that the cleavage of the glutamic acid may be brought about by hydrolysis of the beta-carbon with the production of glyceric acid as follows:



The glyceric acid would then be converted into dextrose.

This method of giving individual amino-acids in diabetes is entirely sufficient to yield information regarding the quantity of sugar production from glycocoll, aspartic acid, serin, and other characteristic building-stones of protein.

On closer consideration it appears remarkable that i-alanin, which is composed of d-alanin found in protein and l-alanin which is only an artificial product, should be completely converted into dextrose. The right-handed and left-handed lactic acid must be equally convertible into dextrose. By what chemical process this synthesis of glucose from i-lactic acid is accomplished is difficult to conjecture. That it should be built up immediately through methylglyoxal and glycerin aldehyde according to the scheme of Wohl,[45] seems difficult to imagine. Certainly the lactic acid can not break up into carbonic acid and alcohol, according to the idea of Stoklasa and Büchner, for these substances do not form dextrose within the organism. On the other hand, if the lactic acid is converted into acetol, $CH_3—CO—CH_2OH$, and finally into formaldehyde, according to the teachings of Walter Löb,[46] the formation of dextrose from the broken fragments would be in accord with modern knowledge. For we have seen that the liver can build glycogen from formaldehyde. One might imagine that this line of varying transformation would be at the sacrifice of some of the potential energy resident in alanin.

Rubner [47] has shown that 28.5 per cent. of the heat value of protein is never utilized by the cells to give them their required energy, but it is lost to the body as waste heat. This power of protein to yield free heat Rubner termed the specific dynamic action of protein. Fats and starches exert a much smaller specific dynamic action because there is only slight heat loss in their conversion into compounds which are directly metabolizable by the cells.

It is on account of this free heat liberated from the different foodstuffs, heat which can not contribute to the necessary mechanics of cell life, that the heat production after food ingestion is greater than during fasting. Rubner conceives the actual energy requirement of the cells to be ever constant under

wide variations in the food supply, and this energy must be furnished to the cells in directly metabolizable compounds. No heat is set free when protein molecules break up into amino-acids,[48] and amino-acids themselves possess the same specific dynamic action as protein.[49] Some heat, however, is liberated when amino-acids are hydrolized to oxy-acids.[50] If one gramme of lactic acid containing 3661 calories were directly converted into one gramme of dextrose containing 3755 calories, there would be little change in the heat relations. If, however, lactic acid was first broken down into formaldehyde and then synthesized into dextrose before it could be used by the cells, energy changes might ensue. However, one gramme of formic aldehyde yields 4010 calories.[51] Hence the energy changes in this intermediate metabolism would be inconsiderable and would not explain the cause of the specific dynamic action of protein.

When one considers that protein in its metabolism yields 28.5 per cent. of its energy content as free heat, and that, besides this, the diabetic eliminates 52.5 per cent. of its energy in the form of urinary sugar, it is evident that the physiologic heat value of protein to the organism in diabetes is only 19 per cent. of its usually calculated value. As if to compensate for this, the protein metabolism rises threefold to fivefold after the administration of phlorhizin to fasting dogs, and Falta [52] has found almost as great a rise in dogs after extirpation of the pancreas. Falta [53] also states that he does not find a higher protein metabolism in human diabetes than normal. Mandel and I, however, in a study of an individual in whom there was complete intolerance for carbohydrates, found that the greatly emaciated patient, when put on a diet containing 7 Gm. of nitrogen, still lost 14 Gm. of body nitrogen besides, an amount which we considered high under the circumstances.

Allard [54] reports a case of a greatly emaciated diabetic man who during fasting excreted between 13 and 14 Gm. of nitrogen in the urine daily. This certainly seems a large amount, considering the condition of the patient.

The total energy requirement of a diabetic is not far different from that of a normal man. Thus, E. Voit [55] calculated

that the heat production of the diabetic patient experimented on by Pettenkofer and Voit in 1867 amounted to 1015 calories per square metre of surface as compared with 1020 calories for a normal individual of similar size. It seems astonishing that a single experiment of Pettenkofer and Voit should have remained for more than forty years a solitary instance of a complete record of the respiratory metabolism in diabetes.

Magnus-Levy,[56] from experiments made on diabetics with the Zuntz respiration apparatus, which determines the respiratory exchange of an individual during a brief interval of time, comes to the conclusion that the total metabolism may be slightly increased in severe diabetes. In phlorhizin glycosuria Mandel and I have shown [57] little or no change in the total heat production from the normal.

Rubner,[58] however, finds that the total metabolism of a dog kept constantly in an environmental temperature of 33 degrees rises from 478 calories on normal fasting days to 510 calories on the days of phlorhizin glycosuria. This 7 per cent. increase in heat production he ascribes to the specific dynamic action of the increased protein metabolism. In this experiment, as in all my own, the fat metabolism remained almost unchanged from the normal, whereas the protein metabolism rose to compensate for the loss of calories eliminated in the urinary sugar.

Sharply discordant with these results are those of Falta, Grote, and Staehelin [59] on depancreatized dogs. In one dog these authors noticed a rise in heat production after extirpation of the pancreas to from 33 to 54 per cent. of the normal amount. But the dog had a small abscess and his temperature ran between 39.4 and 40 degrees after the operation as contrasted with a normal of 38.3 degrees before the operation. In a second dog, abscesses were also found on autopsy, and during one day when the body temperature ran from 38.8 to 39.9 degrees there was an increased heat production of 88 per cent. above the normal, and then during a single three-hour morning period, when the body temperature was low (38.7 to 38.5 degrees), the total metabolism was only 42 per cent. above the normal. This experiment shows the tremendous influence of a fall in body

temperature on metabolism, but it does not show that the morning metabolism of a diabetic dog which had had a temperature of 39.9 the night before is to be considered an uncomplicated criterion of metabolism in diabetes. There might have been an overheating of the cells where metabolism was progressing, even though this was not determinable by the clinical thermometer—an explanation offered by Rubner to explain the rise in metabolism of a fat man in warm air when there was no observable change in his body temperature. From the knowledge at hand the foundations of a diet for a diabetic should, therefore, be one containing about the normal quantity of calories or 35 calories per kilogramme of body weight.

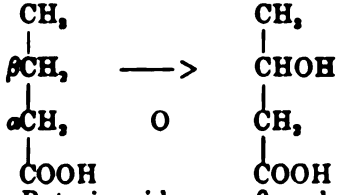
Rosenfeld [60] has pointed out that there is a distinct antagonism between glycogen and fat deposit in the liver. In fasting the quantity of fat in the liver may increase and the fat in the blood also increases. The body fat is transported from its normal repositories in order to feed the tissues. In pancreas diabetes and in phlorhizin glycosuria this condition is intensified so that 40 and even 50 per cent. of the liver solids may consist of fat. Klemperer and Umber [61] have recently reported that of nine persons with diabetes with acidosis seven had lipæmia. Ewing,[62] citing his own work and that of others, finds that the livers of diabetics when they come to autopsy are not fatty in character. This may, perhaps, be explained by the fact that in human diabetics there is rarely a complete loss of power to burn carbohydrate. I have the record of a patient who, revived from coma on administration of bicarbonate of soda, was able to burn ingested carbohydrate in small amount three weeks later without the appearance of any sugar in the urine.

The pre-eminence of fat metabolism in the diabetic as the mainstay of his organism leads to inquiry as to the origin of the fatty acid called beta-oxybutyric acid, and aceto-acetic acid and acetone which are directly derived from it. Whence do these acetone bodies arise? They were at first supposed to come from dextrose, following a chemical process analogous to the butyric acid fermentation of carbohydrates, but it was soon discovered that in normal persons the acetone bodies were espe-

cially found in the fasting state. Many then attributed the presence of acetone to the specific breakdown of body protein, since, when protein was given in the food, the acetone bodies disappeared in the urine. However, Magnus-Levy [63] has reported a case of a boy in coma who eliminated an average of 97.5 Gm. of beta-oxybutyric acid and aceto-acetic acid daily for three days in addition to an unmeasured quantity of acetone in the breath, and during this time the protein metabolism amounted to 90 Gm., of which latter at least 40 Gm. appeared as sugar in the urine. The 97.5 Gm. of acetone bodies in this case could not have been entirely derived from the 90 Gm. of protein, but they must have originated from fat.

Stadelman [64] first pointed out the relationship between the formation of beta-oxybutyric acid and the occurrence of coma. Coma has been compared to the sword of Damocles which hangs suspended over every diabetic. It has been discovered that whenever the organism is thrown suddenly from a carbohydrate regimen to a combustion of fat the acetone bodies appear in the urine. This condition is greatly intensified in diabetes when even the sugar derived from protein is not burned.

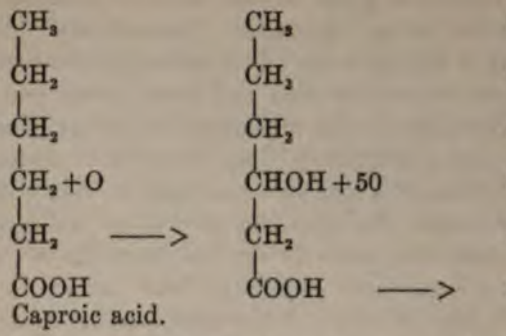
Knoop,[65] through cleverly devised experiments, has shown that the oxidation of fatty acids in the body is effected by an attack on the fatty molecule at the carbon in the beta-position. Thus, the first step in the metabolism of butyric acid would be the oxidation of its beta-carbon atom as follows:



Butyric acid. β-oxybutyric acid.

In a similar manner, caproic acid would first be oxidized at its beta-carbon atom and then on further oxidation would lose two atoms of carbon and be converted into butyric acid, which, in

turn, becomes beta-oxybutyric acid. These reactions may be written as follows:



Caproic acid.

Such, indeed, is believed to be the method of successive oxidation of all the fatty acids, of palmitic acid $C_{16}H_{32}O_2$, of oleic acid $C_{18}H_{34}O_2$, of stearic acid $C_{18}H_{36}O_2$. It is evident that each successive oxidation carries away two carbon atoms and that beta-oxybutyric acid can be produced only from fatty acids having an even number of carbon atoms. Valerianic acid, for example, with five atoms of carbon, can not yield beta-oxybutyric acid. The organism has an apparent preference for fats with an even number of carbon atoms, and each of these fatty acids on their way in metabolism yields a molecule of beta-oxybutyric acid.

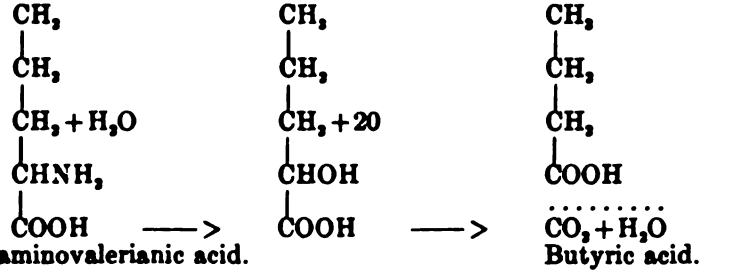
Each molecule of butyric acid can yield one of beta-oxybutyric acid. It has been calculated by Magnus-Levy [66] that 100 Gm. of neutral fat made of stearin, palmitin, and olein may yield 36.2 Gm. of beta-oxybutyric acid. It is, therefore, evident that the higher fatty acids are the more valuable nutriment. Butter, with its high content of butyric acid, largely increases the output of the acetone bodies in diabetes. Fifty to 100 Gm. of butter fat when administered to a diabetic may raise his acetone output four to eightfold.[67] Oleomargarine is to be preferred.

Joslin [68] has shown that oleic acid yields acetone more readily in diabetes than do palmitic and stearic acids.

The story of the formation of beta-oxybutyric acid does not

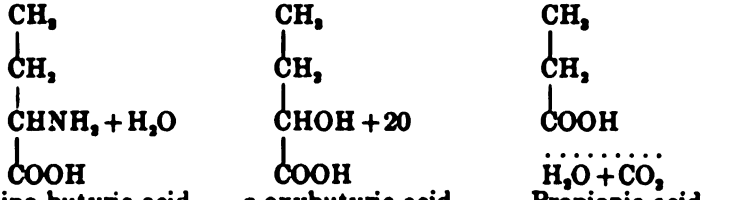
end with the metabolism of fat, for many of the amino-acids of protein yield this acid in metabolism. From the experiments of Embden, Salomon and Schmidt,[68] Baer and Blum,[70] it has been discovered that leucin may yield beta-oxybutyric acid, whereas amino-butyric and normal amino-caproic acids do not. Friedrich Müller, in his Herter lectures two years ago, mentioned the fact that he had administered amino-valerianic acid to a diabetic patient, with resulting increase in the beta-oxybutyric acid excretion.

These statements are all conformant with the idea of a beta-oxidation of fatty molecules. Thus, when alpha-amino-valerianic acid is ingested, it undergoes hydrolysis in the intestinal wall and loses ammonia. Its further oxidation results in the production of butyric acid, which is now oxidized at the beta-carbon. The reaction is as follows:



α-aminovalerianic acid. Butyric acid.

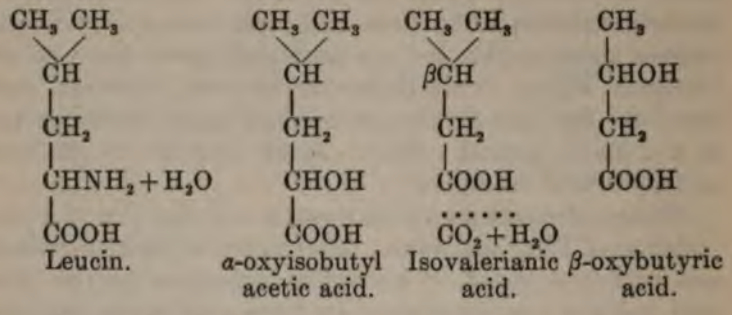
In a similar manner amino-butyric acid and amino-caproic acid would produce, respectively, proprionic and valerianic acids, neither of which is convertible into beta-oxybutyric.



Amino-butyric acid. *α*-oxybutyric acid. Propionic acid.

In the case of leucin, iso-valerianic acid would be the intermediary product, and it has been shown that this fatty acid

with its broken chain is convertible into beta-oxybutyric acid in the organism.



Leucin. α-oxyisobutyl acetic acid. Isovalerianic acid. β-oxybutyric acid.

Amino-acids which form sugar on ingestion, such as glycocoll, alanin, aspartic acid and glutamic acid, do not form beta-oxybutyric acid, but may rather decrease the quantity produced, especially if the sugar formed can burn. This explains why the acidosis in fasting is reduced on ingestion of meat. Baer and Blum [71] gave 10 Gm. of alanin to a dog which received about a gramme of phlorhizin daily. The sugar output was raised from 19.5 to 21.5 Gm. Since we have seen that alanin is completely convertible into dextrose, it follows that much of it must have been burned in the incompletely phlorhizinized dog. Therefore, the acetone excretion decreased and beta-oxybutyric acid disappeared. The profound effect of the ingestion of glutaric acid in reducing sugar and nitrogen output as well as the acetone bodies may find a similar explanation. It is certain that the skilfully planned work of Baer and Blum loses a large part of its significance because of the too small and too infrequent dosage with phlorhizin.

Magnus-Levy [72] gave 11.7 Gm. of beta-oxybutyric acid to a normal dog. This was completely burned. He then gave 11.5 Gm. to a phlorhizinized dog, with the result that there was an increased elimination of 7.6 Gm. of beta-oxybutyric acid and acetone. Since some acetone was eliminated in the breath, it is evident that the animal had largely lost the power to burn ingested beta-oxybutyric acid.

Whatever will materially reduce the metabolism of fat in the body will evidently diminish the source of beta-oxybutyric acid. Such a substance is alcohol. Thus, Benedikt and Török [72] were able to reduce the acetone excretion, as well as that of nitrogen and dextrose after administering alcohol to a diabetic. Stäubli,[74] however, states that alcohol may reduce the tolerance of the diabetic for carbohydrate. Unfortunately, the administration of galactose, of levulose, and of pentoses is of little value in diabetes. In severe cases levulose is largely converted into dextrose or eliminated in the urine. Stäubli finds that ingestion of levulose reduces the diabetic tolerance for dextrose. Brasch [75] finds that the pentoses rhamnose, arabinose, and xylose are not convertible into dextrose in the organism of phlorhizinized dogs; they tend to raise the protein metabolism. Similar results have been obtained in man, and in man pentoses also produce diarrhœa.[76]

On the basis of work on a diabetic and comatose boy weighing 32 kg., Magnus-Levy [77] makes the following computation of metabolism. He purposely assumes a high requirement of energy for a lad of this size, or 50 to 55 calories per kilogramme, which calls for a total of 1600 to 1700 calories. The boy burned 90 Gm. of protein and perhaps 200 Gm. of fat:

90 Gm. protein = 369 calories 200 Gm. fat = 1,909 calories		= 2278
Deduct 97.5 Gm. oxybutric acid, 443 calories Deduct 50 Gm. urinary sugar, 185 calories		= 628
Calories available		1650

Here we perceive an extreme case of diabetic metabolism in which half the energy contained in protein is excreted in urinary sugar and 20 per cent. of that contained in fat is eliminated in the unburned beta-oxybutyric acid.

This, then, is the worst picture of the perverted metabolism in diabetes. Sugar can not burn, fat burns only as far as beta-oxybutyric acid, and as for protein a part of its amino-

acids are converted into sugar and another part into beta-oxybutyric acid, neither of which can be burned.

Rosenfeld has said that fat can burn only "in the fire of carbohydrates." But this is not true. Mandel and I, in our work on a diabetic with a D:N ratio of 3.65 to 1, who had no tolerance for carbohydrates, found a low acidosis as measured by a maximum excretion of 2 Gm. of ammonia, no beta-oxybutyric acid, and a maximum of 0.8 Gm. of acetone per day. On the other hand, Von Noorden [78] and Magnus-Levy [79] report cases in which there was a considerable excretion of acetone bodies in the urine when carbohydrates were burned. For example, one patient eliminated 4.9 Gm. of beta-oxybutyric acid on a day when 40 Gm. of starch were ingested and burned. There are great individual variations. Thus, Stäubli [80] reports concerning a diabetic man whose ordinary mixed diet was changed to one of meat and fat, including 50 Gm. of bread, the whole containing 3200 calories. After ten days of this diet, during which the sugar output remained nearly constant at 100 Gm., the beta-oxybutyric acid fell from 37.5 Gm. daily to nothing. In commenting on his results Stäubli says: "The important factor which causes a more serious condition in the metabolism of a diabetic is the quantity in which carbohydrate is administered in excess of the tolerance for sugar. Damage caused by a continual overworking of the sugar-burning capacity plays a large part in the progress of the disease. The considerable withdrawal of carbohydrates from the diet, even in cases of severe diabetes with high acidosis, exerts an extraordinarily beneficial influence. This can be, in part, explained by the increased ability to burn sugar on account of the conservation of the body's power in this direction. The improvement in the capacity for sugar combustion exerts on its side a beneficial action on the acidosis."

The damage done in severe diabetes by flooding the organism with carbohydrates is illustrated by a diabetic individual who had kept on a restricted diet at my advice, but on the recommendation of a consultant was given a large quantity of

carbohydrate; this resulted in onset of coma, which proved fatal.

The great individual variations as regards the presence of the acetone bodies seem to warrant the opinion which I expressed in a discussion on acidosis at Washington two years ago, that one may assume the existence of a specific beta-oxybutyric acid ferment analogous to the ferment which breaks down the sugar. Such a ferment would split beta-oxybutyric acid, thereby performing one of the last offices of cleavage of the fatty molecules. Injury to this ferment may be complete or partial even as in the case of the sugar ferment, but damage to one does not necessarily involve proportionate damage to the other.

The elimination of beta-oxybutyric acid from the system is furthered by the administration of alkalies. Stäubli [80] reports a diabetic who eliminated 34 Gm. of beta-oxybutyric acid daily when the diet contained 60 Gm. of sodium bicarbonate. This excretion fell to 17 Gm. on a diet which was free from alkali, and then rose to 45.2 Gm. on return to 60 Gm. of bicarbonate. Such treatment with alkali is highly beneficial, for, as Magnus-Levy observes, the diabetic does not die in coma because of the neutralized acid which is eliminated in the urine, but rather on account of that which is retained in the body which neutralizes the alkalis of tissue and of body fluids.

Bedard, Pembry and Spriggs [81] find that the blood of the diabetic in coma still has considerable power to hold carbon dioxide in spite of the acidosis. They explain that the reduction of carbon dioxide in the blood is due to the extra ventilation brought on by dyspnœa, and that the dyspnœa is the result of acids rendering the respiratory centre especially sensitive to carbon dioxide and other stimulating substances.

I have purposely traced the doctrine of diabetic metabolism through its most acute manifestations. There are, however, countless variations from the extreme conditions. The hope for the diabetic lies in dieting. His carbohydrate tolerance must be determined. Mandel and I [82] have recommended that the patient be put on a strict carbohydrate-free diet and the

D:N ratio of the second day of the diet be determined. If the ratio be 3.65 to 1 it is the "fatal ratio" and represents a complete intolerance for carbohydrates. A lower ratio represents hope for the patient. Following this method we found the "fatal ratio" in one patient who died a month later. In another case of a young man revived from coma and placed on a meat and fat diet the D:N ratio fell steadily from 2.8 to 1 on the second day until the tenth day, when it was 0.34 to 1, and after three weeks the urine was free from sugar even after the ingestion of small quantities of carbohydrates. Two years later the ratio was 2.8 to 1 after a week of strict meat and fat diet, which indicated a less favorable outlook, although the patient maintained his weight and went about his usual occupation. A year later he died in coma.

A splendid piece of metabolism work on a diabetic man was published a year ago by Allard [82] from Minkowski's clinic at Greifswald. I have taken the liberty to rearrange the figures. The experiment was accomplished on a man weighing between 51 and 52 kg., who entered the hospital in a state of extreme emaciation. During residence in the hospital his weight improved. He was given various diets and allowed to fast, and account was kept of the nitrogen, dextrose, ammonia, beta-oxybutyric acid, and acetone eliminated in the urine. The results may thus be tabulated:

EXPERIMENT BY ALLARD
FROM MINKOWSKI'S CLINIC AT GREIFSWALD

Date.	Diet.	Period, hrs.	Urine, c.c.	N Gm.	D Gm.	D:N	NH_3	β-oxybutyric acid.	Acetone.	Total acids.
Feb. 22	Meat and fat	24	5130	32.9	105.6	3.25	5.2	17.34	5.88	23.22
Feb. 23	Fasting	24	5395	14.0	30.6	2.11	3.1	2.95	1.82	4.77
Feb. 24	300 Gm. nutrose	24	3070	27.6	95.5	3.47	4.0	2.86	1.68	4.55
Mar. 20	Meat and fat	24	4730	19.2	71.8	3.75	5.0	15.14	6.73	21.87
Mar. 21	Fasting	12	2815	6.5	23.0	3.67	1.7	2.34	1.47	3.81
Mar. 21	200 Gm. butter	12	2335	4.6	25.2	5.48	1.8	5.66	1.79	7.45
Apr. 6	Meat and fat	12	2195	15.7	52.0	3.34	2.6	8.43	4.05	12.48
Apr. 7	Fasting	24	5155	13.6	16.4	1.21	3.4	6.28	2.98	9.26
Apr. 11	200 Gm. butter	24	4905	13.0	40.1	3.08	4.0	16.03	5.67	21.71

This experiment shows the beneficial action of a fasting day on the acidosis of a diabetic. Thus, on February 22, when the patient ingested meat and fat, the total acetone bodies amounted to 23 Gm. in twenty-four hours, and on February 23, during

fasting, they fell to 4.8 Gm. The ammonia was also greatly reduced. The D : N ratio fell from 3.25 to 2.11. On February 24, 300 Gm. of nutrose (a sodium compound of caseine) were given, without raising the acidosis above the fasting amount, but the D : N ratio became 3.47. On March 20, on a meat-fat diet, the acidosis was again high and the D : N ratio was 3.75. The next day of fasting the acetone bodies fell very largely and the D : N ratio remained at 3.67. These represent the maximum ratios in diabetes as I understand them. On March 21, 200 Gm. of butter were given, causing a rise in the acidosis and a rise in the ratio to 5.48. Whether this high ratio is due to nitrogen retention or to the conversion of glycerin into dextrose can not be determined. In the light of other evidence it is not probable that any of the fatty acids ingested were converted into dextrose. A fasting day on April 7 showed a low acidosis and a D : N ratio of 1.21. An improvement in the power to burn dextrose had, therefore, taken place, although it appears remarkable that this was not accompanied by a decreased elimination of nitrogen and acetone bodies, as contrasted with the amounts excreted on former fasting days when the D : N ratio was higher. On April 11 the improvement in the ratio was largely nullified by the ingestion of 200 Gm. of butter. The excretion of acetone bodies rose from 9 to 22 Gm. and the D : N ratio rose to 3.08. Apparently a high acidosis lowers the tolerance for carbohydrate, just as a large ingestion of carbohydrates lowers the tolerance for beta-oxybutyric acid. This experiment does not justify the assumption of Mandel and myself, that the "fatal ratio" once established will continue throughout life. I have presented this table to indicate to you a high type of modern clinical work.

There is no cure for diabetes. Only dieting relieves the sufferer. Of the results of dieting, Dr. Falta, speaking with the authority of Von Noorden's great clinic, and of his own good work, will address us at the next Harvey Society Lecture.

Administration of extracts of the pancreas and the ferments of yeast are without effect. Falta[41] has injected subcutaneously a normal dog's serum into a dog with pancreas diabetes

without changing the D:N ratio, and again he has introduced the lymph of a normal dog, drop by drop, into the femoral vein of a diabetic dog without result.

Physicians call on the laboratories for a cure, but there is no cure. All that the laboratories can furnish are indices whereby relief may be rendered. It is easy enough to give the results of the gross activities of many million millions of cells in terms of so many grammes of sugar or of nitrogen or of beta-oxybutyric acid, but it is not easy to gain access to a mystery which is at present the hidden secret of microscopic particles. But we must not despair. The great physiologist, Johannes Müller, stated that no man would ever measure the rapidity of the nerve impulse, and ten years later his pupil, Helmholtz, measured it. It is to the increasing number of young men who are attracted by the scientific or intellectual side of medicine that the world hopefully turns for relief from the miseries of its diseases.

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THE THERAPY OF DIABETES MELLITUS*

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IN the lecture which I had the honor to deliver a few weeks ago before the New York Academy of Medicine, I explained that human diabetes mellitus was a disease of highly complicated nature. I then took the stand that we should not seek in the pancreas the sole cause of the disease, but rather that a rôle is also played therein by other organs which elaborate internal secretions, and especially by the nervous system. In most general terms I defined the diabetic disturbance of metabolism as a lack of equilibrium between carbohydrate mobilization and carbohydrate combustion, arising from insufficiency of the pancreas or from overactivity of the chromaffin system, or from both causes together. Apart from the rare cases in which grave pancreatic disease has been found, we are at present ignorant of the ultimate cause of this disturbance of metabolism, and accordingly a causal therapy, the ideal therapy in every disease, is to-day out of the question. Symptomatic therapy seeks, in the first instance, to combat the most prominent symptom, the excretion of sugar and its results.

Theoretically two possibilities exist:

1. To increase the efficiency of carbohydrate metabolism. Conceivably this might be accomplished by increasing the internal secretion of the pancreas by the implantation of a new organ—until now a pious hope—or by the use of pancreatic secretion. Recently Zuelzer has reported such experiments, but to-day they do not possess practical value. Further, it may be expected that a diminution of the excessive carbohydrate mobilization should increase the utilization of sugar. This might be accomplished by checking the nervous system. The

* Delivered November 28, 1908.

success which occasionally accompanies the use of sedatives may come about in this way. Unhappily such successes have been slight.

2. To diminish the amount of carbohydrate metabolism, thereby giving the diseased organ or organs the opportunity of recovering. This may be brought about by diminishing the amount of the food, especially of the most effective sugar-formers. This is the theoretical foundation of the dietetic therapy of diabetes mellitus, which has thus far been regarded as the sovereign means of treatment.

Before turning to the therapeutic measures which aim to improve the carbohydrate metabolism, I wish to consider a small group of cases which manifest a marked disease of the pancreas, for these often require a special therapy. When in such cases pancreatic disease has proceeded so far that there is no longer an adequate secretion of pancreatic juice into the intestine; or when, the more common event, lithiasis causes complete obstruction of the pancreatic duct, characteristic disturbances of absorption arise. These concern chiefly protein and fat. The very voluminous stools contain great quantities of neutral fat; microscopically the picture is mainly muscle-fibres and fat droplets. In extreme cases the fat may flow out of the anus in an oily mass and stiffen into a butter-like paste. Salomon has pointed out that, in doubtful cases, by feeding large quantities of butter, such stools may be caused and the diagnosis established.

These patients are in a grave condition; protein and fat are very imperfectly absorbed, while the carbohydrates are excreted, unutilized in the urine, as grape sugar. Therefore the patients are starving and they actually live on their own tissues and rapidly lose flesh. In such cases therapy is of great avail in replacing the pancreatic secretion. Von Noorden first used the raw pancreas of the ox, but this preparation quickly becomes obnoxious, and accordingly pancreatic extract has been substituted for it. Since the acid gastric juice diminishes the activity of pancreas preparations, tablets hardened with tannin have been employed, in the expectation that they would not be

disintegrated before reaching the intestine. This preparation, under the name of pancreon, is, however, not always active. According to our experience, the most successful preparation is the pancreatin of the Rhenania factory, in doses of 10 Gm. daily. At the same time, however, the diminished alkalinity of the intestine must be repaired. According to Von Noorden, this may best be accomplished by the use of calcium carbonate, which is less readily absorbed from the stomach than sodium bicarbonate. Further, it may be mentioned that emulsified fat (milk-fat or egg-yolk) is much better utilized than fat in other forms. With the help of this therapy in such cases the body-weight may often be readily increased, and for years together tolerable conditions for the life of the patient may be established.

I turn now to the disturbances of the carbohydrate metabolism. The chief end of dietetic therapy is here the depression of glycosuria. Of course, we must not attend to this factor alone; complicated acute infectious diseases may often cause a very large increase in the formation of the ketone bodies; further, chronic infectious diseases, as, for instance, tuberculosis, may often, if not always, prevent a strict dietetic treatment. In light cases of tuberculosis, however, an improvement in the tuberculous process is often to be observed with the disappearance of sugar from the urine. Indisposition of the gastro-intestinal tract, often the result of a long-continued protein-fat régime, sometimes occupies a prominent position. However, the occurrence of acidosis may prohibit a decrease in the carbohydrate of the diet. This matter will be discussed later.

I wish now to present a question of fundamental importance. On what grounds do we seek to bring about a disappearance of glycosuria? Obviously by the excretion of sugar the food loses just so much of its value. For instance, in an extreme case, 500 Gm. of sugar may be excreted in twenty-four hours; if this patient receives an ordinary diet, which yields 2500 calories daily, he is losing through the excretion of sugar in the urine 2000 of these calories; accordingly the patient is living

on his own body and rapidly loses flesh. Such cases of extreme glycosuria are, of course, exceptional. In cases in which 100 Gm. of dextrose are daily excreted, the loss of energy is only 400 calories, and these may readily be replaced by the equivalent quantity of fat, for instance, by 50 Gm. of butter. Further, the circumstance that the protein-sparing carbohydrates are the substances here involved is not of essential importance. In my publications numerous instances are cited which show that often, in the most severe cases of diabetes with enormous excretion of sugar, a comparatively small addition of calories may prevent a loss of protein; and, indeed, that a tendency, thus far difficult to explain, to a retention of protein exists. The salient point accordingly depends on another circumstance, which may be formulated as follows: Glycosuria is a result of hyperglycæmia. In this connection there arise from the more recent investigations in Von Noorden's clinic therapeutic considerations of great importance. Liefman and Stern have shown that in long-continued cases of diabetes mellitus, even with relatively slight excretion of sugar, very considerable hyperglycæmia may be displayed; and that, after the disappearance of sugar from the urine in such cases, it may be a very long time before the sugar content of the blood has sunk to its normal level. Apparently after long-continued hyperglycæmia, the kidneys lose their high degree of sensitiveness to slight increase in the sugar content of the blood. As an example, I present the following case recently observed in the clinic: With a daily sugar excretion of 5 Gm. the blood sugar amounted to 0.36 per cent.; on the twenty-third day after the disappearance of sugar from the urine it still amounted to 0.123 per cent., instead of the normal content of 0.085 per cent.

We are accustomed to refer many, indeed most, of the secondary phenomena in diabetes to hyperglycæmia: the lancinating pains, furunculosis, pruritis, the falling out of the teeth, the failure of hearing, the premature cataract, the impotence, the vulnerability of the tissues, and the early arteriosclerosis with gangrene. But the significance of hyperglycæmia is far greater than this, in that long-continued hyperglycæmia increases the

disturbance of metabolism, thereby establishing a vicious circle! We understand accordingly why all these results of hyperglycæmia, and one especially, namely, gangrene, are to be found even in apparently very mild cases of diabetes. These individuals may excrete a few grammes of sugar daily, but they may have had diabetes and accordingly hyperglycæmia for fifteen years. Thus we perceive the desirability, even in cases of the diabetes of old age, of not being content when the excretion of sugar has been reduced to a few grammes, but of insisting on complete disappearance of sugar. The understanding of many characteristics of tolerance is to be found in these relations. Thus we often perceive that even severe cases in youth, which may only with difficulty yield sugar-free urines, possess for a remarkably long time no tolerance for carbohydrates; indeed, we have recently observed such a case in the clinic, which, nine days after the disappearance of sugar from the urine, showed a sugar content of the blood of 0.21 per cent. So long, therefore, as the blood sugar has not been reduced to its normal level no tolerance is to be expected. In cases in which the careful administration of carbohydrate in small quantity produces an immediate excretion of sugar, it is well to remove carbohydrate entirely from the diet during several weeks. In the above-mentioned case it took two months, after the urine was sugar-free, to establish a slight tolerance of approximately 10 Gm.

I turn now to the question how glycosuria is to be combated. There is in the literature an extensive accumulation of facts on the influence of various foodstuffs on glycosuria. I myself, with several collaborators, have been occupied with this problem during the past five years. The results of the older as well as the more recent investigations indicate that the disturbance of metabolism in diabetes is of a very complex nature, and that thus its intensity is subject to many factors. Each case has its peculiar characteristics and requires a special study; schematic treatment here would be quite out of place. Before determining on a plan of treatment, therefore, it is necessary to obtain an estimate of the character and intensity of the

disease. In order to accomplish this, it is advisable to place every new patient for a period of three days on a test diet of known composition. At our clinic the following test diet is used: 250 grammes of meat, 150 grammes of butter, 4 eggs, 300 grammes of vegetables with low carbohydrate content. In addition, tea, bouillon, coffee, about 4 decilitres of light white wine and 75 grammes of white bread, divided through the day in three equal portions are given. This diet contains approximately 16 grammes nitrogen, 50 grammes carbohydrate and about 2400 calories. Cases with severe acidosis and with signs of beginning coma are, of course, excepted; for these the carbohydrate content of this diet would be too low and therefore dangerous.

The sugar excretion on this diet can vary considerably. I have noted here some of the types:

	First day	Second day	Third day
Case 1—			
Dextrose, grammes	30	10	5
Acetone	0	0	0
Case 2—Dextrose, grammes—			
Titration	90	60	40
Polariscope	85	60	25
Acetone	+	++	+++
Case 3—			
Dextrose, grammes	20	40	50
Case 4—Dextrose, grammes—			
Titration	70	70	70
Polariscope	45	45	45
Acetone	+++	+++	+++

Nitrogen average = 15 grammes.

In Cases 1 and 2 we may assume that the patients had previously taken more carbohydrate than is contained in the test diet. Case 1 is a very mild one; the patient, in all probability, will become sugar-free on further treatment. Patient 3 probably has been previously on a strict diet. Patient 4 had probably been before on a diet similar to the test diet. In this manner we gain an approximate idea of the dietetic habits of the patient previous to beginning treatment, irrespective of the patient's own account. Furthermore, by means of this test

diet we can obtain a quick diagnosis of the degree of ketonuria (acetonuria).

I take this opportunity to point out that in severe cases, with large amounts of beta-oxybutyric acid in the urine, polarization is quite inadequate in the quantitative determination of sugar. This is undoubtedly self-evident; nevertheless, it is often overlooked in practice, as well as in laboratories and pharmacies. Polarization before and after fermentation has the drawback that we must await the completion of the fermentation. Fehling's method requires considerable practice. It is practical, therefore, to employ a modification of Fehling's method, in which the unreduced copper is titrated back by means of potassium iodide and sodium thiosulphate, in acid solution. The estimation by this method can be performed in ten minutes. It is not infrequent in severe cases, especially under strict diet, to observe differences of 30, 40, yes, occasionally even 50 grammes between the figures obtained by titration and by polarization. This difference is also a measure of the amount of oxybutyric acid excretion, a better one than is obtained by the determination of the amount of ammonia, since the latter is influenced by the sodium therapy and is dependent on the degree of protein metabolism.

From the difference between the titration and polarization and from the intensity of the acetone test and ferric-chloride reaction, we may obtain during the three days' test diet an idea of the degree of ketonuria. Thus we see in Case 1 that the patient becomes almost sugar-free without the appearance of acetonuria. This is a very mild case. In Case 2 the sugar falls off rapidly, too, but the ketonuria rapidly increases and warns us to be careful. Case 4 is stamped from the beginning as a severe one by the character of glycosuria and ketonuria.

In judging a case further, the intensity of the glycosuria is of great importance. We consider, in this respect, chiefly the third day of test diet, as it may be assumed that by this time a condition approaching equilibrium has become established. A sure opinion of the intensity of the glycosuria can be obtained only when we know, not merely the carbohydrate content of the

food, but also its other constituents, since the sugar may be derived from various sources. In Cases 1 and 2 the sugar excretion is less on the third day than the carbohydrate intake. In Case 3 intake and output are about equal; in Case 4 the sugar excretion is some 20 grammes higher than the carbohydrate intake.

Next in importance as a source of sugar is the oxidized protein. Furthermore, there are, indeed, cases in which the sugar excretion is so great that even the protein destroyed does not suffice for an explanation. In these cases we have no alternative for the present but to assume the formation of sugar out of fat. We observe occasionally, also, that a very large fat intake will increase the sugar excretion. However, these cases are relatively rare. In the great majority of all diabetics, the sugar excretion is determined by the carbohydrate and protein content of the food.

In the fourth case, mentioned above, the sugar excretion exceeds the carbohydrate intake by 20 grammes. We have here a so-called negative carbohydrate balance of 20. This terminology is, however, inexact, because it overlooks the protein metabolism. The figure 20 has an entirely different meaning according as 10 or 30 grammes of nitrogen are simultaneously excreted in the urine.

One obtains, therefore, a better conception of the conditions through the ratio D : N. In Case 4 this factor equals:

$$\frac{70-50}{15}=1.33.$$

One must not, however, assume that in this case only 20 grammes of sugar are derived from the protein metabolism. On the contrary, it is much more probable that, in severe cases, the protein and carbohydrate are implicated according to their sugar value in the formation of urinary sugar. If we assume the sugar value of albumin to be 80 per cent., then for every gramme of nitrogen in the urine there are 5 grammes of dextrose. The sugar value of the metabolized material accordingly

amounts to the carbohydrate intake plus five times the urinary nitrogen, and the coefficient of excretion is:

$$\frac{D \times 100}{\text{carbohydrate} + 5\ N.}$$

In Case 4 this coefficient amounts to:

$$\frac{70 \times 100}{50 + (5 \times 15)} = \frac{7000}{125} = \frac{56}{1}$$

That is, 48 per cent. of the sugar value of the food was excreted and 52 per cent. was assimilated. If the excretion coefficient rises over 100, and in consequence the ratio D:N rises over 1 to 5, then this indicates, in my opinion, sugar formation from fat.

Since we know the exact composition of our test diet, we can calculate the intensity of the sugar excretion according to this formula with accuracy. Since nitrogen retention frequently occurs in diabetes, it is desirable to determine the nitrogen content of the urine of the third day. By our test diet we have, then, determined the intensity of the glycosuria and ketonuria. If, in addition, the age, occupation, surroundings, the duration of the diabetes and possible complications are considered, we may arrive in the space of three days, with comparatively simple means, at a fairly clear idea of the intensity of the disease, which is of great advantage in forming a plan of treatment. A definite opinion, however, can be reached, it is true, only in the course of further treatment. It is dependent on the answers to the following questions: Can the patient be made sugar-free? Can a sugar tolerance be established? What is the course of the ketonuria?

We enter, then, into the second phase of the treatment; we attempt to render the patient sugar-free. In the mild cases it usually suffices to strike out the bread from our test diet. Thus it is to be expected, in Cases 1 and 3, that the urine will be sugar-free after a few days. In the severer cases it is necessary, in addition to the withdrawal of carbohydrates, to limit

the protein intake. This is readily to be understood in view of what I have said before. The sugar value of our test diet amounts to $15 \times 5 + 50 = 125$. Let us assume that the patient's sugar tolerance in Cases 1 and 3 is at 90; after the withdrawal of carbohydrates from our test diet we obtain a sugar value of 75, which is below the limit of the present tolerance—the glycosuria ceases. In Case 2 let us assume the limit of tolerance to be 50. We reach a level below the limit of tolerance then only if, in addition to the withdrawal of carbohydrate, we diminish the protein content of the food by at least 5 grammes of nitrogen. This method is of considerable advantage also in the severe cases. In Case 4 there are, during the time of the test diet, 70 grammes of dextrose in the urine. We now withdraw the carbohydrates and find, after two or three days, 36 grammes. We could thus assume that the ability to assimilate the carbohydrate had diminished. However, if we take into account the sugar value of the protein, we obtain, in the first period, a total sugar value of 125 in the diet and a coefficient of 56; in the second period, a total sugar value of 75 in the diet and a coefficient of 48. Thus there has been little change. It is true that such conditions are found only in very severe cases. In the great majority of all cases, the power of utilization rises rapidly with the diminution of the total dietary sugar value, for less sugar passes through the body in twenty-four hours, the glycæmia decreases and recovery begins. The consideration of the total sugar value, therefore, always gives us a much clearer conception of the conditions than the carbohydrate content of the food alone. The same is true of the carbohydrate tolerance. I propose, therefore, that the tolerance be also expressed in terms of the total sugar value of the diet.

In practice we proceed as follows: If the withdrawal of carbohydrate alone does not suffice, we decrease the nitrogen content of the food to about 8 grammes and increase the amount of butter to about 200 grammes. The patient should not lose weight during the process of making his urine sugar-free. The sugar value of this food would amount to only about

50. If this also proves insufficient, it is advisable to introduce one or two days of vegetable diet; or, according to Naunyn, one day of fasting; or else one tries an oatmeal cure, to be discussed later.

At this stage of the treatment a very close watch on the ketonuria is necessary. The formation of the ketone bodies depends, as you know, on the lack of combustion of carbohydrates. Ketonuria may be produced even in the normal person by means of inanition or by an exclusive protein and fat diet. The fact that much higher grades of ketonuria appear in diabetes is easily understood, since here there is also a failure to consume the sugar arising from the katabolism of proteins. Von Noorden has pointed out that diabetics may show very different degrees of ketonuria; although they are on the same diet, they may eliminate the same amount of sugar in the urine and assimilate, therefore, the same amounts of carbohydrates. We have often observed this phenomenon in patients taking our test diet. We can assume with a fair degree of certainty that those with a higher degree of ketonuria have had it for a longer time than those with a small amount of ketonuria. I am led to this conclusion by the fact that it is necessary to give more carbohydrates to suppress a ketonuria already existing than to prevent the occurrence of ketonuria (Satta). We ought, therefore, to try either to prevent the occurrence of ketonuria or to avoid an increase of one that is already present. Unfortunately this is not possible in most cases, if we wish to render the patient sugar-free. If on the test diet there is a marked ketonuria, and in particular a large amount of beta-oxybutyric acid in the urine, it is best to reduce the carbohydrates very gradually. The appearance of a considerable degree of ketonuria, however, need not cause anxiety, provided that one prevents acidosis; that is to say, the storing up of ketones in the body. Acidosis, once present, is often hard to get rid of and is dangerous under all circumstances. The fundamental point in the treatment, therefore, is to hinder the occurrence of acidosis. This is accomplished by the administration of alkalies, which render the ketone bodies capable

of excretion in the urine. We should, therefore, always give sodium bicarbonate during the period of withdrawal of carbohydrates. In cases with a well-marked ketonuria one often sees that, with the beginning of the alkaline treatment, the difference between the figures for sugar obtained by titration and by polarization rises immediately and remains increased for several days; a sign that beta-oxybutyric acid is being flushed out of the body. The reaction of the urine affords an excellent sign as to whether you have got the better of the acidosis. If it becomes alkaline, you may be sure that you have neutralized the acids. In severe cases, 40 or 60 grammes of the alkali or more are necessary, and in diabetic coma more than 100 grammes are often insufficient. If a marked ketonuria is present, it is better not to begin with small doses, but to saturate the body with alkali at once. As soon as the urine has become alkaline, the dose may be gradually decreased, just sufficient being given to keep the urine slightly alkaline. We usually replace a part of the sodium bicarbonate with the citrate of soda.

In cases with a high degree of acidosis, the administration of alkalies alone is not enough; we must endeavor to influence the formation of ketone bodies directly. Large doses of alcohol decrease the formation of ketones, but only to a slight degree. All other non-carbohydrate substances are either without effect or their action is dubious. The only efficacious substances are the carbohydrates. If, however, we give large amounts of these, we increase the hyperglycæmia and run the danger of damaging the power to oxidize carbohydrates, which is already defective. On the other hand, a reduction in the carbohydrates may cause a fatal coma; here we are truly between Scylla and Charybdis. In these cases it seems to me very important to reduce the food to a low point; for both fat and protein cause the production of large amounts of ketones, as has been shown by the excellent investigations of Allard, from Minkowski's clinic. The introduction of a "hunger day" in such cases has reduced the ketones in the urine to a third or less of what they were before. If, now, to the fasting patient fat or protein was

given, the amount of oxybutyric acid rose at once 20 grammes and more. It is, therefore, best to give a very low diet, containing from 8 to 10 grammes of nitrogen and not more than 150 grammes of fat and, in addition, 100 or 150 grammes of carbohydrates in the form of fruit, milk, oatmeal, and so forth. As soon as one has decreased the formation of the ketone bodies and has provided for their prompt excretion by means of the administration of alkalies, one may for a short period also decrease the amount of carbohydrates (by means of two or three "vegetable days," with 50 grammes of carbohydrates), in order to produce at least a temporary decrease of the hyperglycæmia. Similar tendencies are seen also in the modern treatment of gout and acute nephritis, where we give the diseased organs time for recuperation by means of purin-free and non-nitrogenous diets, respectively.

When, in spite of careful treatment, the first signs of coma appear, prompt action is imperative. The patient becomes sleepy, loses his appetite, and complains of a sense of oppression in the chest; frequently there is a decrease of the excretion of beta-oxybutyric acid, and showers of casts appear in the urine. Such patients show almost constantly a marked degree of lipæmia, even when fasting. One can easily show this by centrifugalizing a small amount of blood in a U-shaped capillary tube; the serum then appears milky. Very large doses of alkali are necessary here, in order to remove the enormous amounts of acid which are contained in the blood and tissues; 100 grammes of sodium bicarbonate dissolved in a large amount of an alkaline mineral water are given during the course of the day. The diet should be easily digestible and consist almost exclusively of carbohydrates; it may contain 50 to 100 grammes of levulose. If the patient is no longer able to swallow, the levulose may be given subcutaneously, dissolved in a litre of physiologic salt solution. The best method, however, is the intravenous infusion of a litre of 4 per cent. soda solution. Sometimes the patient comes out of the coma even during the infusion. Last spring I saw a patient who was alive three months after this procedure. I do not, however, know of any

instance of recovery from a second attack of well-marked coma under this treatment.

Let us now return to the treatment of those patients who have become sugar-free through the withdrawal of carbohydrates, with or without the reduction of the proteins. This is the third stage of the treatment, in which we are to keep the patient free from sugar, and then to determine his tolerance and regulate his mode of living. It is advisable to follow Naunyn's suggestion and keep the patient two weeks to the diet on which he has become free from sugar. Then, in a mild case, one may begin at once with the addition of bread, which may be increased every three or four days until the limit of tolerance is reached. Then you may give half the amount tolerated, introducing variety in the bill of fare by means of fruit, cream, vegetables rich in carbohydrates, various kinds of bread, and so on.

In those severe cases, in which the reduction of the proteins is necessary to cause the disappearance of sugar from the urine, one should at first increase the nitrogen in the diet up to 12 or 14 grammes. Afterward, three or four weeks after the disappearance of sugar, carbohydrates may be added. In practice it is very important not to rely too closely on the amount of carbohydrates in various foods, as shown by the tables of equivalents. This is true of all forms of diabetes, from those which can not be rendered free from sugar down to the very mild ones. Different diabetics do not show the same degree of tolerance for different kinds of carbohydrates; one must try out each form of carbohydrate in the individual case. In this connection, we often see that levulose is borne in many light cases better than glucose; for this reason fruits rich in levulose are much preferable to those containing grape-sugar. Under long-continued use of levulose, however, the tolerance for sugar usually sinks rapidly. Furthermore, I have seen cases in which there was a special sensitiveness toward levulose. The same is true also of milk; a few diabetics will not tolerate milk. I have recently seen a case in which administration of 250 grammes of milk increased the glycosuria markedly and for a period

of days. Further, it is an interesting fact brought out by our investigations with various sorts of sugar that maltose always causes the greatest increase in the glycosuria. This agrees with the empirical fact that diabetics do not tolerate beer well; it is best to forbid it. This point, however, is of less importance in America than in Germany. Of the substitutes for bread, I think the most important is *Luftbrot*, a very porous gluten bread, of which a loaf the size of two fists weighs only 30 or 40 grammes. It contains very little carbohydrate in proportion to its bulk, and is not intended to replace bread, but to serve as a means of administering cheese and butter. It is well to be very cautious in the use of the various substitutes for bread. Both the public and the physician are often deceived by the name "diabetic bread," and even by the analyses which are given on the label. I myself would not order any of them unless reliable analyses were at hand. Finally, I have to mention the special milk for diabetics which is prepared by many firms, and especially preserved fruits containing little sugar.

Especial care is necessary in the treatment of those cases in which the withdrawal of carbohydrates and the reduction of the proteins is not sufficient, and the sugar disappears from the urine only after the introduction of "hunger days" or "oatmeal cures." In such cases, even weeks after the disappearance of glycosuria, small additions of carbohydrates, or even an increase of the proteins, cause the reappearance of sugar in the urine. I have already called attention to the fact that here hyperglycæmia may remain for a long time after the disappearance of sugar from the urine. I have seen in recent years three such patients, two of whom were young people of 16 and 20 years, and the third a man of 35, who, when he came under my care, had not been free from sugar for a year. In the case of the 16-year-old boy, even an addition of butter to the diet caused the appearance of traces of sugar. The three patients had at first well-marked ketonuria, and one of them excreted 22 grammes of beta-oxybutyric acid. I kept these patients over three months on a diet free from carbohydrates and containing only a small amount of protein, and then added

half a *Luftbrot* and later preserved fruits containing little sugar. Six months afterwards they were still free from sugar, able to do their work, and the ketonuria had disappeared. Such rigorous treatment demands great energy on the part of both patient and physician. When, however, we consider that in such cases with the reappearance of sugar and hyperglycæmia the disease usually takes a rapidly downward path, and, therefore, the acquirement and maintenance of a condition free from sugar is a question of life and death, the self-denial of the patient and the labor of the physician are amply repaid.

All the measures against glycosuria previously mentioned had the common property of reducing the sugar value in the diet. From this principle the following carbohydrate cures differ widely. The milk and potato cures I can dismiss with a few words. The first diminishes the glycosuria only when diabetic patients, formerly over-nourished, are insufficiently nourished on strict milk diet. Indeed, in many cases, in spite of under-nourishment, milk diet can even increase glycosuria. The potato cure is considered by nearly all experienced physicians to be without value. On the other hand, I must go somewhat more into detail regarding the oatmeal cure, since it is of both theoretical and practical value. As you know, this treatment was first recommended by Von Noorden at the meeting of the German scientists in Carlsbad. In an article shortly to appear I shall describe fifty cases, of which I have personally observed eight in my wards; the others Professor von Noorden has reported to me from his private practice. From all the results in these cases I can single out only a few important points. First, I must describe our present method of giving the oatmeal cure. After several days of strict diet come two vegetable days, then three oatmeal days, and lastly two vegetable days. On each oatmeal day the patient receives 250 to 300 grammes of American oatmeal, prepared with an equal amount of butter and divided into five meals; and, in addition, black coffee, wine, a little brandy and, when necessary on account of diarrhœa, a few drops of tincture of opium. Frequently when the first trial has been unsuccessful, we have

obtained good results from an immediate repetition of the oatmeal days following the last vegetable days. After the vegetable days we give strict diet with very little (diminished) protein. I may express the results in short as follows: In a certain proportion of the cases (about 10 per cent.) the success can be called really remarkable. These are severe cases with ketonuria which it had formerly been impossible to make sugar-free either by completely removing the carbohydrate from the diet, or by a low protein diet, or by a strictly vegetable diet. In these cases the sugar and ketone bodies disappeared from the urine during the oatmeal days or the following vegetable days, and the patient could subsequently be kept sugar-free. For a second group of cases the sugar entirely or almost wholly disappeared, but later reappeared on the return to a protein diet. In a third group of severe cases the glycosuria remained high or even increased; nevertheless, the influence on the ketonuria was remarkable. Finally, we have seen a few very severe cases, in which there was no assimilation of the oatmeal and, therefore, no influence on the ketonuria.

For a thorough understanding of the effect of the oatmeal the following points must be noted: The sugar value of an oatmeal day is 200 grammes, 175 from the carbohydrate and about 25 from the protein of the oatmeal. The sugar value of our test diet is 125 grammes, that of the carbohydrate-free diet only 80, that of the diet with restricted protein is about 50, and that of a vegetable day is still less. Therefore, if in some cases during the preliminary vegetable days 20 or 30 grammes of sugar are excreted daily, and then during the oatmeal days the sugar entirely disappears, this result is truly astonishing; and it furthermore can be readily understood how through the assimilation of such an enormous quanitity of carbohydrate the ketonuria often diminishes from about 40 grammes almost to nothing, and the output of ammonia becomes normal. Let us take, however, a case in which the average sugar output increased from 30 grammes on the vegetable days to 40 or even 60 grammes on the oatmeal diet; even in such cases the marked effect on the ketonuria is readily understood, for, on each vege-

table day, of the entire sugar value only 20 grammes are assimilated; whereas, on each oatmeal day, from 140 to 160 grammes are assimilated. We must, then, consider as the essential result of the oatmeal cure the fact that we are enabled to bring about the assimilation of a considerable quantity of carbohydrate, and thus to diminish the ketonuria without increasing too rapidly the hyperglycæmia. What is very remarkable in this connection is the fact that, with other carbohydrates similarly used, we do not get the same result.

In my report I shall mention a case in which 30 to 40 grammes of dextrose were excreted daily during the oatmeal cure, while on a diet consisting of potatoes, milk, zwieback, fruit, etc., an average of 150 grammes of sugar appeared in the urine. In another case, a diet of wheat flour was given between two periods of oatmeal diet. The difference in the sugar excretion was almost as great as that in the case mentioned. The most important indication for the oatmeal cure, therefore, is the presence of ketonuria; and the fact that some apparently hopeless cases also become sugar-free is a welcome additional occurrence. A further indication for this cure is in cases in which the digestion is impaired by a meat and fat diet which has been too long-continued.

Some authorities have belittled the advantages of the oatmeal cure. Naunyn, for example, believes that the apparent good effect on the glycosuria is brought about by a fermentation of the greater portion of the oatmeal in the intestine. He uses as an argument the results of the investigation of his pupil, Lipitz, who found the intestinal bacteria increased in numbers during the oatmeal cure. This objection, however, does not hold. In the first place, Lipitz's method of quantitative estimation of the bacteria is not reliable. Second, there is practically no clinical evidence of a pronounced intestinal fermentation—no tympanites and no fermenting stools. Third, if there were much fermentation, as Naunyn claims, the effect on the ketonuria could not be explained, for the fermentation products of carbohydrate, since they can not again unite to form sugar in the body, should rather increase the ketonuria, as, for

instance, in the form of low fatty acids. The fourth and most striking point is with regard to the protein metabolism. The nitrogen output amounts, in successful cases, on oatmeal days, to only 5, 4, or even 3 grammes, and, indeed, is sometimes less than the so-called minimum nitrogen determined by Landergren in normal individuals, kept for several days on a practically nitrogen-free diet, rich in carbohydrate. Such low nitrogen outputs during the oatmeal cure are possible only when the carbohydrates are really assimilated, and not merely fermented in the intestine. In such cases also we find regularly an increase in body-weight of two kilos or even more in three or four days. The final test of our assumption is to be found in those few cases in which the oatmeal is not assimilated at all, as shown by the enormous output of sugar while on this diet, for there is no effect on the ketonuria and the nitrogen output amounts to 8 grammes or even more daily.

The cause of this remarkable effect of oatmeal is as yet unknown. That it is not the limited amount of protein in the diet is shown by the fact that Von Noorden formerly added large amounts of vegetable protein to the oatmeal porridge, deriving, however, good results. Furthermore, this assumption can not explain why other carbohydrates do not produce the same result when given in the same way. The argument that the cause lies in the molecular structure of the oat starch also seems weak. Hiss has seen good effects from the use of oat extracts. It may be true that there is an extractive substance in oatmeal which stimulates the internal secretions of the pancreas.

The great variety in symptoms and severity as shown in diabetes in human beings demands many varieties of treatment. In all but the very mild cases the diabetic needs the constant supervision of his physician. Special care, however, must be taken when we wish to make diabetics with ketonuria sugar-free. The treatment of such patients in their homes is possible only if they are very intelligent, and it necessitates great firmness of character. In severe cases it is possible to make them sugar-free only in a hospital. Furthermore, in moderately severe cases a stay for a time in a hospital is advisable, par-

ticularly during any determination of the patient's degree of tolerance. A course of the waters at Carlsbad, Vichy, Neuenahr Marienbad, Tarasp and other spas is often remarkably beneficial. The advantage of such a course is, according to Von Noorden, shown by the fact that during it the patient can often be made sugar-free or can establish a high degree of tolerance on a less rigorous diet than otherwise would be necessary. In extremely severe cases the patients should not be sent to these resorts, but directly to a hospital. A very important element in the treatment is a liberal amount of carefully regulated exercise. Severely affected patients, however, must avoid over-exertion, which often increases the glycosuria markedly, and may do actual harm.

Obese patients with a mild diabetes can, to advantage, be carefully treated for their obesity by restricting the number of calories in the diet; but, in severe cases, it is not advisable to attempt to reduce the body-weight. In cases in which gout is a complication, one must consider carefully the purin content of the diet.

Finally, in regard to the prognosis, the patient's age, environment, and circumstances, and any complications, must be considered, as well as the severity of the glycosuria and ketonuria. I can not now go more into detail regarding prognosis, except to say that the occurrence of diabetes in early years does not preclude a complete cure. There are such patients who have been made sugar-free and have remained so, and are able again to follow their regular occupations. In these cases it is, however, always to be feared that, under unfavorable circumstances, such as excesses, over-exertion, or excitement, the sugar may reappear. In this connection the character of the patient is very important. Moderation, self-control, and steadfastness are essential. I regard such patients as cured, although the predisposition to diabetes is still present. It is only in the same sense that we are able to consider a tuberculous patient cured.

ANAPHYLAXIS*

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ANAPHYLAXIS (*ana*, against, and *phylax*, guard, or *phylaxis*, protection), also called hypersusceptibility, supersensitiveness, is a condition of unusual or exaggerated susceptibility of the organism to foreign substances. The word "anaphylaxis" was introduced by Richet to describe a contrary condition to prophylaxis. As we now regard the phenomenon, the word is a misnomer, for we look upon the condition of hypersusceptibility as a distinct benefit and advantage to the organism. In fact, protection against a large class of infections depends on an altered power of reaction, *i.e.*, hypersusceptibility or anaphylaxis.

This state may be congenital or acquired, and is specific in nature. The condition of anaphylaxis may be brought about by the introduction of any strange protein into the body. Hypersusceptibility to proteins that are non-poisonous in themselves may readily be induced in certain animals.

An animal may be in a condition of hypersusceptibility and immunity at the same time. The two conditions are closely interwoven; the latter is often dependent on the former. Von Pirquet advises that the term "immunity" be limited to indicate the condition of complete resistance in which no clinical reaction occurs, when poisons such as diphtheria, tetanus, etc., are introduced into the organism. He suggests the term "allergie" to indicate conditions of acquired immunity associated with anaphylaxis.

In the case of vaccinia the reaction to a primary "take" appears after an incubation period of four days. In a secondary vaccination the period of incubation is shortened and

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the clinical reaction lessened. In other words, the power of the organism to react has changed. This increased power of immediate reaction protects the individual. There is no absolute immunity in this class of diseases; the prophylaxis depends on the anaphylaxis.

Allergie then, as the word indicates (*allos* change, and *ergon* action), is an altered power of the organism to react. When this power of reaction is increased we say that the body is hypersensitive or in a state of anaphylaxis.

The tuberculin and mallein reactions are well-known instances of anaphylaxis. These substances are not poisonous when introduced into a healthy individual, but the tuberculous individual is anaphylactic to tuberculin, and an individual suffering with glanders is in a state of hypersusceptibility to mallein.

A clinical instance of anaphylaxis is the hypersusceptibility of some individuals to pollen—hay fever. The best studied instance of experimental anaphylaxis is that produced in the guinea-pig by the injection of a foreign protein; for example, horse serum, egg-white, milk, etc. Especial study has been made of the anaphylactic action of the blood serum of the horse, partly because that serum is so much used in serum therapy.

It has long been known that the blood of certain animals is poisonous when transfused or injected into certain other species. Many instances might be cited showing that the blood serum of an animal of one species has poisonous properties when injected into an animal of another species. But the blood serum of the horse apparently lacks such poisonous action. Very large quantities of the blood serum of the horse may be injected into man, rabbits, guinea-pigs, and many other animals without serious inconvenience except occasionally a slight reaction at the site of inoculation.

In a certain proportion of cases the injection of horse serum into man is followed by urticarial eruptions, joint pains, fever, swelling of the lymph-nodes, œdema, and albuminuria. This reaction, which appears after an incubation period of

eight to thirteen days, has been termed by Von Pirquet and Schick the "serum disease."

In exceptional cases, sudden death has followed an injection of horse serum in man.

We have shown that ordinarily horse serum is a comparatively bland and harmless substance when injected into certain animals; but these animals may be rendered so susceptible that an injection of horse serum may produce death or severe symptoms. For example, large quantities of horse serum may be injected subcutaneously, into the peritoneal cavity, into the brain, or directly into the circulation of a guinea-pig without apparently causing the animal the least inconvenience. If, however, a guinea-pig be injected with a small quantity, say 0.004 c.c., of horse serum and, after the expiration of a certain interval, again injected with horse serum the result will probably be fatal. The first injection of horse serum has sensitized the animal in such a way as to render it very susceptible to a second injection of horse serum.

A certain time must elapse between the first and the second injections before the animal becomes susceptible to a second injection. This period of incubation is from seven to twelve days, and corresponds suggestively with the period of incubation of the serum disease, which Von Pirquet and Schick place at eight to thirteen days.

Guinea-pigs may be sensitized with exceedingly small quantities of horse serum. In most of our work we used quantities less than 0.004 c.c., and we found in one instance that 0.000,001 c.c. of horse serum was sufficient to render a guinea-pig susceptible.

Wells has recently shown that guinea-pigs may be sensitized with 0.000,000,05 of a gramme of egg albumen freed from the other proteins of egg-white.

It also requires very small quantities of horse serum, when given in a second injection, to produce poisonous symptoms. One-tenth of a cubic centimetre injected into the peritoneal cavity is sometimes sufficient to cause death; 0.1 c.c. subcutaneously may cause symptoms, while much smaller amounts

given into the brain or directly into the circulation may be fatal.

At first we thought that diphtheria antitoxin had some relation to this phenomenon; we are now able to state positively that it has nothing whatever to do with the poisonous effects of horse serum; further, that diphtheria antitoxin in itself is absolutely harmless. The toxic action which we have studied is caused by a protein in normal horse serum and is entirely independent of the antitoxic properties of the serum.

REVIEW OF LITERATURE.

Early in the last century Magendie[92] found that rabbits which had tolerated two intravenous injections of egg albumen without any ill effects immediately succumbed to a further injection made after a number of days. Later workers with precipitins have frequently found that some of their animals died suddenly during the course of treatment from no apparent cause, while what really happened was that they were in a state of anaphylaxis to the foreign proteid. Other analogous instances may be found scattered throughout the early literature.

In 1888 Arloing[7] expressed the opinion that pathogenic micro-organisms secrete soluble substances which influence the organism in such a way that at a later infection it succumbs more quickly. The organism is robbed of its natural protecting bodies through the first process. Arloing evidently had in mind that the power of reaction of an organism could be changed.

In 1891 Courmont[36] studied this question with reference to the tubercle bacillus and the subject was further pursued by the Lyons School with staphylococci, streptococci, *B. pyocyaneus*, and other bacteria.

Brieger,[30] in 1895, immunized a goat to a high degree against tetanus. The blood and milk of this animal contained large quantities of antitoxin. The animal, however, died of tetanus.

Knorr,[82] in 1895, studied this unexpected phenomenon more closely and found that guinea-pigs developed an increasing sensitiveness to tetanus toxin after repeated sublethal doses.

Uhlenhuth,[148] in 1897, worked on the relative toxicity of blood serum in health and disease. He found that intravenous injection into rabbits was not reliable and therefore used subcutaneous injections into guinea-pigs. He found that the normal sterile blood serum of man, sheep, hog, rabbit and cattle even in small doses (about 0.5 c.c.) produced infiltration in guinea-pigs when injected subcutaneously and necroses when given in large quantities. Twenty c.c. of these serums (10 to 15 of cattle serum) is sufficient to cause the death of guinea-pigs. Normal horse serum is not toxic, even in large doses (20 c.c.); at most it only produces slight infiltrations which are rapidly absorbed.

Hericourt and Richet,[77] in 1898, in studying the effects of eel serum on dogs, found that they were not able to immunize them against the serum, but that on the contrary there was an increasing sensibility to it, so that finally the dogs died.

Courmont,[47] in 1900, found that a guinea-pig might support an intraperitoneal injection of a pleuritic effusion of about one-fifteenth its weight (30 c.c. for a 400 Gm. guinea-pig), without fatal effect, whereas doses very much smaller (several c.c.) given in repeated injections under the skin or into the peritoneum caused œdema and death.

Von Behring and Kitashima,[153] in 1901, repeating some of Knorr's work, found a similar instance in a horse immunized against diphtheria. This curious phenomenon, sometimes seen with bacterial toxins, was called hypersusceptibility and was spoken of as a paradoxical reaction. Von Behring and Kitashima then made their experiments on guinea-pigs. Von Behring looked on the hypersusceptibility as purely histogenetic.

Portier and Richet,[109] in 1902, found that if dogs were given a very small dose of a glycerin extract from the tentacles of actinia, and then in fifteen or twenty days given a second small dose, the animals quickly succumbed. The dose given was so small as to cause no symptoms in a normal animal. They proposed the word "anaphylaxis" to indicate hypersensitiveness to a poison.

Arthus,[9] in 1903, studied the effect of repeated injections of horse serum on rabbits and found that if a rabbit were given repeated injections of horse serum at some days' interval it caused, even in small doses, results which, according to the number of the previous injections and methods of injection, were local or general, benign or grave.

Von Pirquet,[167] in the end of 1902, noticed that the symptoms following a second injection of horse serum in a child occurred on the same day and concluded therefrom that the current views on the period of incubation must be wrong. He believed that the organism must be changed through the production of antibodies and that the incubation period was the terminus of the production of these antibodies.

Von Pirquet and Schick,[167 168] in 1903, stated that an organism treated with a foreign serum was distinctly changed from one that had not been so treated. They believed that the strange serum did not work directly on the organism, but that there must be a reaction product or antibody. At the second injection the reaction might occur at once. This they called the "immediate" reaction. When the period of incubation was shortened they called it "accelerated" reaction. Von Pirquet and Schick found a number of analogies between these phenomena and the incubation periods of certain diseases, and mention specifically vaccination and the tuberculin reaction. Hypersusceptibility was shown in the case of injection with horse serum as well as tuberculin in those sensitized with tuberculosis.

1904.

Wolff-Eisner,[179] toward the end of 1904, worked on the question of hypersusceptibility in the sense of Pfeiffer's endotoxins. From this starting point he handled the subject broadly and published his views. He studied the lysis of pigeon's blood corpuscles and human spermatozoa after the first and second injections into the peritoneal cavity of a guinea-pig. He observed that guinea-pigs always succumbed at subsequent injections. He explained this phenomenon on the ground that

poisonous substances or endotoxins contained within the cells are freed when the cell membrane is dissolved. This solution is produced through a lysin formed by the previous treatment.

Detre-Deutsch,[43] in 1904, studied the question why persons with syphilis do not show, on secondary inoculation, a primary lesion. He carried out experiments on guinea-pigs by reinfecting them with tuberculosis and showed the difference between the course of the first and the second injection, which he called "superinfection." His explanation of the acute action of the second injection coincides with Koch's addition theory of the tuberculin reaction.

1905.

Richet,[117] in studying two poisons (congestine and thalassin) extracted from actinia, found that if a very small dose, which caused practically no symptoms in a dog, was followed after twenty-two days by another small dose the animal became very sick or quickly died. He found that not only did his animals remain anaphylactic for a long time, but that it was necessary that a certain time elapse between the first and the second injections for the development of this increased susceptibility to the foreign protein. He further states that anaphylaxis is a new phenomenon which has never before been named or described, and that it is of the same order as that produced by the injection of tuberculin in an animal suffering from tuberculosis.

Von Pirquet and Schick,[170] in a monograph on the serum disease, describe in detail this syndrome which sometimes follows injections of horse serum in man. They show that the symptoms of this disease when caused by a second injection may either appear at once (the immediate reaction) or after a shortened period of incubation (the accelerated reaction). From these clinical observations Von Pirquet and Schick draw original and far-reaching conclusions. They show the relation of these clinical observations to the phenomenon of hypersusceptibility, and indicate the importance of these facts in general pathology. They draw attention to the analogy to

the tuberculin reaction as a well-known instance of hypersusceptibility. Von Pirquet and Schick believe that the serum reactions give a possible explanation of the period of incubation of infectious diseases and finally conclude that the immunity caused by vaccinia and a group of infections is due to the power of immediate reaction acquired by the organism.

During Ehrlich's visit to America in 1904, Theobald Smith told him that guinea-pigs which had been used in testing the potency of diphtheria antitoxin became acutely sick or died from a subcutaneous injection several weeks later of several cubic centimetres of normal horse serum. Ehrlich gave the problem to Otto,[104] who worked out many essential features of the phenomenon, to which he gave the name of the "Theobald Smith phenomenon."

Otto showed what is now well known to be the result of a second injection of horse serum into guinea-pigs. He demonstrated that the diphtheria poisons played no part in the phenomenon. He found, however, that guinea-pigs first treated with mixtures of diphtheria toxin and serum were more susceptible than those treated with serum alone. Otto showed further that immunity to the poisonous action of the serum injection might be acquired by repeated injections of large amounts of serum at short intervals. He demonstrates that this hypersusceptibility bears no relation to the specific precipitins. Finally, he discusses the relation of the Theobald Smith phenomenon to the cases of reinjection in man and cites instances of alarming symptoms following the second injection of antitoxic horse serum.

Pfeiffer [108] confirmed Uhlenhuth's observations that certain sera, for example hog and sheep serum, caused gangrene of the skin and subcutaneous tissue when injected into a guinea-pig. On the other hand, the serum of horses and rabbits caused practically no reaction. Pfeiffer demonstrates that through repeated injections of normal serum it is possible to immunize the animals against this necrotic action and also that the serum of immunized animals is able to protect animals of the same species. He further confirms Uhlenhuth's explanation of the

phenomenon by assuming that the serums contain a haptin in the sense of Ehrlich's side-chain theory. He believes the necrotic action to depend on and to be identical with hæmolysis.

1906.

Rosenau and Anderson give the results of their studies, with special relation to anaphylaxis in guinea-pigs. They show that a single injection of horse serum is harmless for normal animals. Horse serum is, however, poisonous to a guinea-pig which has previously been injected with horse serum. The period of incubation was determined to be about ten days. The poisonous principle appears to act on the respiratory centres. The heart continues to beat after respiration ceases. It is shown that the toxic action of horse serum bears no relation to diphtheria. The poison is not toxin. Diphtheria antitoxin plays no part in this poisonous action and in itself is harmless. The effects of chemical, physical and electrical influences on the toxic and sensitizing principle are considered. It is shown that guinea-pigs remain susceptible a very long time and that very small quantities (in one instance 0.000,001 c.c.) of horse serum are sufficient to render guinea-pigs susceptible.

It is shown that an active immunity against the toxic action of horse serum may readily be established by repeated injections of horse serum at short intervals into a guinea-pig. Rosenau and Anderson did not succeed in transferring this immunity in the blood serum or body juices to another guinea-pig. It therefore appears that the immune body, if one exist against the toxic action of horse serum, is not free in the blood or body juices.

It is shown experimentally that the guinea-pig may be sensitized by feeding with horse serum or with horse meat.

It is further shown that susceptibility to the toxic action of horse serum is transmitted from the mother guinea-pig to her young.

The specific nature of the phenomenon is shown. The opinion is expressed that the substance which sensitizes the animal is identical with that which later poisons it. The sub-

stance must, however, first cause a reaction in the organism, resulting in the production of antibodies. How man may be sensitized is considered in relation to the cases of collapse and sudden death following the injection of horse serum.

Anderson [2 3 4] found that female guinea-pigs could transmit to the same offspring hypersusceptibility to horse serum and immunity to diphtheria toxin. This fact, Anderson states, is of great importance in testing antitoxic serums and necessitates care in the selection of breeders for guinea-pigs to be used in serum work.

Vaughan [150] advanced the theory that the first injection of the strange proteid is broken up into components, one of which is toxic, but that the animal is not poisoned because this breaking up takes place slowly. The cells, however, learn from this lesson how to break up the complex molecule, so that when more of the strange proteid is introduced at the second injection it is violently rent asunder, quickly liberating large quantities of the toxic principle of the complex molecule.

McClintock and King,[95] as a result of their work, conclude that the sensitizing action of horse serum given by the mouth is not nearly so great as when given subcutaneously or intraperitoneally.

Remlinger [113] found, in experiments on dogs, rabbits and guinea-pigs, an entire absence of anaphylaxis.

In his first series of experiments, he gave his animals normal horse or sheep serum or antidiphtheric or antitetanic serum; one month later, they received from 5 to 20 c.c. of a mixture of equal parts of antirabic sheep serum and emulsion of fixed virus.

In the second series of experiments, the animals were first treated with a mixture of fixed virus and antirabic sheep serum, and six or eight weeks later were given the serums above mentioned. None of the animals in either series showed any immediate symptoms, though two guinea-pigs and four rabbits died in five or six days.

These results, so contrary to all of the anaphylactic work, may be concerned, in part, with the question of specificity.

Von Pirquet [157] explains the theoretical considerations of the accelerated reactions with special reference to vaccinia. The different characters of the areolas and the papular reactions are described and the conclusion drawn that vaccination does not cause an absolute immunity, but changes the power of reaction of the organism in such a way that it reacts sooner. Thus, when the organism is reinfected the process comes to a conclusion in a short time.

In a brief note Von Pirquet [155] suggests the name "allergie" (*allos* change, and *ergon* reaction) to indicate that condition of immunity achieved through an altered power of reaction. The close association between immunity and hypersusceptibility and the relation of these processes to the so-called endotoxins is discussed. Von Pirquet advises that the term "active immunity" should be limited to those conditions in which the introduction of a foreign substance into the organism causes no clinical reaction, that is, in which there is a complete lack of susceptibility. He discusses whether this is produced through alexins (natural immunity), through antitoxins (active or passive immunity against diphtheria and tetanus), or whether it is caused through a form of adaptation. (Wassermann and Citron.)

Von Pirquet [156] concludes that the accelerated reaction in vaccinia is a specific reaction between the virus of cowpox and an immune or allergic organism. The accelerated reaction depends quantitatively upon the amount of the virus that is introduced. From the theoretical standpoint Von Pirquet expresses the belief that the accelerated reaction is caused by the coming together of the vaccine virus with the antibodies in the allergic organism and that the precipitins are not concerned.

1907.

Gay and Southard [55] found in guinea-pigs dying from the second injection of serum and in those which had severe symptoms and were later chloroformed, what they considered characteristic lesions. Considerable hemorrhages, rather

definitely localized, are the characteristic gross lesion. The hemorrhages may be in one or several organs, gastric hemorrhages being especially frequent. Microscopically, there are in addition to the naked-eye hemorrhages, minute, interstitial and oozing hemorrhages. They also claimed to have found fatty changes in voluntary muscle fibre, heart muscle fibres, and in nerve fibres.

Their explanation of serum anaphylaxis in the guinea-pig is substantially as follows: There is a substance in horse serum (anaphylactin) which is not absorbed by the guinea-pig tissue, is not neutralized, and is eliminated with great slowness from the body. When a guinea-pig is injected with a small amount of horse serum the greater part of its elements are quickly eliminated; the anaphylactin remains and acts as a constant irritant to the body cells, so that their activity for the other elements of horse serum is greatly increased. At the end of two weeks of constant stimulation by the anaphylactin a condition is arrived at in which, if the cells are suddenly presented with a large amount of horse serum they are overwhelmed in the exercise of their increased assimilating functions, and functional equilibrium is so disturbed that local or general death may occur.

In a second paper Otto [105] demonstrated that guinea-pigs might be sensitized by injecting them with the blood serum of sensitized guinea-pigs, and further brought out the important point that guinea-pigs sensitized by such a transfer reacted within twenty-four hours. He believes that the first injection results in a weakening or depressing of the portions ("rests") of the antigens which are in the body and thus an apparent hypersusceptibility results. The duration of this hypersusceptibility depends on the amount of serum injected the first time.

Besredka and Steinhardt [22] studied with much care certain features of hypersusceptibility to horse serum in guinea-pigs; they note that the French serums are much less toxic than those used by Otto in Frankfurt and the serums used by Rosenau and Anderson. Besredka and Steinhardt had a mor-

tality of about 25 per cent. when 5 c.c. of serum were given intraperitoneally at the second injection, whereas death was the rule in our experiments under similar conditions. Most of their work was done with doses of 0.05 to 0.25 c.c., given directly into the brain, which either killed or caused grave symptoms in susceptible guinea-pigs. Besredka and Steinhardt lay stress on the production of "antianaphylaxis," which we termed "immunity." They found that a single injection of serum, given into the peritoneum of a sensitized guinea-pig, quickly conferred immunity to a subsequent injection of 0.25 c.c. into the brain; in one case the antianaphylaxis was present one and a half hours after the injection into the abdominal cavity. They were unable to demonstrate any protective properties in various organs of immune guinea-pigs.

Besredka and Steinhardt[23] found that guinea-pigs could be put in a state of antianaphylaxis by the injection of horse serum into the brain as well as into the peritoneal cavity. They consider it a phenomenon of the same order as the disintoxication *in vitro* of the tetanized brain by antitetanic serum. They found that guinea-pigs could not be sensitized by intracerebral injection.

They think that their results seem to indicate that the phenomena of anaphylaxis and antianaphylaxis are similar to the precipitating and absorbing actions which govern the relation of colloids among themselves.

Besredka[15] concludes that the toxicity of therapeutic serums may be measured by means of intracerebral injections into sensitized guinea-pigs. Measured in this way, different serums show a wide range of toxicitiy, the fatal dose varying from $^1/_4$ to $^1/_{128}$ c.c. This toxicity resides in the serum and not in the cellular elements.

The serums of horses living under apparently the same conditions have about the same toxicity; individual variations are rare and of little importance. The difference in the toxicity of serums appears to be due, in the first place, to their origin; and, in the second place, to their age. Serums are hypertoxic on the day of bleeding, and gradually lose their

toxicity. This loss, rapid at first, becomes gradual after the tenth day. All therapeutic serums should be considered toxic within two months of bleeding. In a general way, all serums that excite grave anaphylactic phenomena in doses of 0.0625 to 0.05 c.c., and *a fortiori*, above this amount, should be considered toxic.

Besredka finally states that the technic and dosage by the intracerebral method is rapid, simple, and not expensive.

Besredka,[19] in a preliminary note, states that serum heated to 100 C. has lost all of its toxicity for sensitive guinea-pigs, but still possesses some vaccinating properties; while serum heated to even 120 C. is still sensitizing. He believes there are two substances in horse serum: (*a*) the *sensibilisinogène*, which is thermostabile and gives birth to the *sensibilisine*, which create the anaphylactic state; (*b*) the *antisensibilisine*, which is thermolabile and, being an antibody, combines with the *sensibilisine* when the two come in contact. It is the sudden union of these two at the nucleus of the nerve cell which causes the symptoms of anaphylaxis, especially marked when the second injection is given into the brain.

Besredka [16] considers that there may be two ways in which the toxic property of serum for sensitive guinea-pigs can be altered. These are *directly* on the serum, and *indirectly* on the animal itself. Of all the various *direct* means, such as chemical, physical or biologic, only heating the serum to high temperature is of avail. The toxic property is progressively decreased by heating from 76° C. until at 100° C. it disappears. He also finds that the immunizing power of the heated serum follows the same curve as the toxic property. The repeated heating of the serum three or four times at lower temperatures, such as 50° C. or 60° C. diminishes the toxic property three or four times.

As *indirect* means, he cites the use of serum as a preventive measure, either during the preanaphylactic period or after the period of incubation. He states that ether narcosis prevents the appearance of anaphylactic symptoms, but that neither morphine nor extract of opium has any influence on the appearance of the symptoms.

In a further contribution to the subject Rosenau and Anderson [130 131] studied particularly the relation of anaphylaxis to immunity. They express the opinion that profound chemical changes, perhaps in the central nervous system, are probably produced by the first injection of a strange protein. Many details concerning the sensitizing and toxic principle were studied. In addition to extending and confirming their work on the specific nature of the phenomenon they made further observations on the relation of various physical influences and chemical substances on the reaction. Among other things they brought out the fact that proteids extracted from the bacterial cells and injected into guinea-pigs produced, on the second injection, the same train of symptoms as in the case of serum anaphylaxis. It was found that in certain instances the hypersusceptibility manifesting itself from injections of these bacterial extracts left the animal immune to the corresponding infection.

Rosenau and Anderson [132] later demonstrated the specific nature of anaphylaxis by showing that guinea-pigs may be in a condition of anaphylaxis to three protein substances at the same time. For instance, a guinea-pig may be sensitized with egg-white, milk, and horse serum and subsequently react in a brief period of time to a second injection of each one of these substances. It may be sensitized by giving these strange proteins either at the same or at different times, in the same or in different places, or by injecting them separately or mixed. The guinea-pig differentiates each anaphylactic-producing protein in a perfectly distinct and separate manner. The animal acts as though susceptible to three separate infectious diseases. The conclusion is therefore drawn that the phenomenon of anaphylaxis is specific and the belief expressed that the work indicates that chemical changes rather than morphologic alterations lie at the basis of this phenomenon.

Von Pirquet [133] again clearly defines his conception of allergie and points out that a complete immunity does not exist. For example, in revaccination there is an immediate though slight clinical reaction and it is on this condition of hypersusceptibility that immunity depends. The convenience

and advantages of the cutaneous vaccination for studying the problem and for diagnosis, especially in tuberculosis and other infectious diseases, are discussed.

Von Pirquet's [163] admirable clinical studies on vaccination and vaccinal allergie are fully described by him in a brochure published in 1907. The clinical side of primary vaccinations are first considered and then the clinical side of secondary vaccinations are pictured and discussed. He gives a clear picture of accelerated reaction in all its details largely by the use of curves and tables. Finally, Von Pirquet's conception of allergie in relation to the early vaccinal reactions is explained. The theories of hypersusceptibility are fully discussed and their relation to the phenomenon of vaccination is considered. Von Pirquet concludes in part that these vaccinal characteristics were more or less known by the old vaccination physicians but, up to now, have been forgotten.

The new points discovered by Von Pirquet are especially the morphological, the early reaction and the method of presenting them in curves. The observation that the normal time of reaction is gradually changed to the "immediate" reaction in secondary vaccinations is also new. Von Pirquet further brings forward his conception of the cachectic reaction and the differentiation between the papular and the areolar reactions. He insists on the presence of antibodies which lie at the foundation of accelerated reaction. An analysis of the local symptoms is divided into two processes: one, the growth of the infective principle; and second, the production of antibodies in the organism which explains the accelerated area-reaction.

Richet [122] gives a general review of the subject of anaphylaxis and also some very interesting work on anaphylaxis produced by a substance obtained from the *Mytilus edulis*. He found that the blood of a dog sensitized by this substance, when injected into an untreated dog, sensitized the animal two days later to an injection of the extract.

He thinks that anaphylaxis is due to the presence of a toxicogenic substance, non-toxic of itself, but producing a

poison by reaction with the second injection of the extract. In support of this view, he states that a mixture of the serum of a sensitized dog and of the extract *in vitro* is more toxic than the extract alone.

Richet [120] reports further experiments on the production of anaphylaxis with an extract prepared from a mussel (*Mytilus edulis*).

The experiments were made on dogs, especial study being made of the production of vomiting in the anaphylactic animal.

Vaughan and Wheeler [152] summarize their work to date on the poisonous and non-poisonous portions obtained by splitting proteins. They believe the poisonous group to be an essential constituent of all the protein molecules. It is the chemical nucleus and is regarded as a protein body the chemical structure of which remains unknown. It owes its poisonous action to the avidity with which it combines with certain groups in the molecules that constitute the cells of the respiratory centre. The protein molecules of bacteria may thus be broken up, sensitizing the body. The poisonous group, when set free, induces the symptoms of disease and death. Protein susceptibility and immunity are different manifestations of one and the same process; both depend on the development in the animal body of a specific proteolytic ferment. They believe the non-poisonous portion of split proteins to be the immunizing or sensitizing haptophore. The sensitization results in the development of a specific proteolytic ferment. These reactions are specific. The development of a specific zymogen results from an alteration in the atomic arrangement within the protein molecules. Vaughan and Wheeler give a complete bibliography of the work of Vaughan and his pupils on this subject.

Currie [29] studied the effect of repeated injections of horse serum in persons admitted for treatment in the Glasgow Fever and Smallpox Hospital at Belvidere. He concludes that it is apparent from the facts detailed by him that repeated injections of horse serum induce symptoms of supersensitization

in man, but it is also apparent that the same facts lend no countenance to the suggestion that the death of persons suffering from diphtheria is to be apprehended as the result of repeated injections of antidiphtheria serum.

Goodall [64] gives observations on ninety patients who had received two injections of horse serum; of these, 43.4 per cent. gave either an immediate or accelerated reaction.

Nicolle [100] found that guinea-pigs were not susceptible to the necrotic action induced by repeated injections of horse serum, as is the case in rabbits. He also found that daily injections or "spaced" injections, after the method of Arthus, did not induce a high degree of hypersensibility in guinea-pigs.

Bienenfeld [25] has studied the leucocytes in the serum disease, and comes to the conclusion that the injection of large quantities of serum has a twofold effect on the number of leucocytes. Immediately following there is a leucopenia, and following this a leucocytosis. The bibliography contains fifty-two references to the literature on this phase of the subject alone.

Friedemann and Isaacs [55] report experiments on the metabolism of proteins introduced into the organism, through channels other than the gastro-intestinal tract. The relation to immunity and hypersusceptibility is discussed. Experiments on dogs and goats on nitrogen elimination, after the injection of homologous and heterologous serums, are considered. It is shown that dogs given intravenous injections of 40, 80, or 200 c.c. of egg-white soon die with characteristic symptoms including great muscular weakness and paralysis of respiration.

Heilner [74] considers the effect of large amounts of foreign blood serums introduced into the body by the mouth or subcutaneously. He concludes that the introduction of such foreign serums, and also perhaps other proteins, into the circulation calls forth a certain effort which ordinarily is not present and, further, reports the fact that rabbits are able to withstand, without apparent harm, a single injection of very great

quantities of foreign serums—as much as one-eighth of the body weight.

While studying the subject of anaphylaxis in Rio de Janeiro, Vasconcellos[149] found that the guinea-pigs used in Rio, when given a sensitizing dose and, after the usual time, given a second injection, did not show any grave symptoms. He tested the Rio guinea-pigs with the Rio serum as well as with the serum from the Pasteur Institute at Paris, and from *Merck's* and obtained similar results in each case.

In order to see whether this failure of the Rio guinea-pigs to show the reaction of hypersensibility was due to the race of the guinea-pigs, he obtained guinea-pigs from Argentina and found them very susceptible.

He considers that the race of the guinea-pigs plays a considerable rôle in the reaction to a second injection of horse serum.

Weil-Halle and Lemaire[175] prepared an antiserum by injecting horse serum into rabbits and bled the animals when the horse serum as such had disappeared from the blood. Untreated guinea-pigs and rabbits were injected with varying amounts of this antiserum, under the skin of one thigh, and at the same time, with varying amounts of horse serum, under the skin of the opposite thigh. They found that varying with the proportion of the two serums injected, anaphylactic symptoms were produced, either local or general. When local symptoms were produced, they were always at the site of the injection of the antiserum. They believed that the intensity of the reaction seemed to depend on the relation between the quantities of the two serums; when the quantities approach unity the lesions are more local; when they are wide apart, the general symptoms are more common.

Remlinger,[114] having found in previous experiments that he was unable to produce anaphylaxis in dogs, rabbits and guinea-pigs by the use of rabic virus and sheep serum, endeavored to determine whether this was peculiar to those substances, and if the same results would be obtained with antidiphtheria and antitetanus serums. With these serums, by

repeated injections, he obtained no reaction in dogs. In rabbits and guinea-pigs, he obtained the local reactions and only exceptionally the general reactions.

When guinea-pigs and rabbits were given an injection of a mixture of serum and toxin and later injected with normal horse serum, some of the guinea-pigs showed symptoms.

Kinyoun [79a] found that guinea-pigs sensitive to horse serum did not react if they were injected with the blood of a horse which had received several injections of human blood; but that the serum of a horse which had been rendered hæmolytic for human red cells was more toxic than normal serum.

He states that the toxic action is modified by the amount of the sensitizing dose so that the susceptibility is decreased as the amount of serum given at the first injection is increased until one-tenth of the body weight is reached, when they do not respond at all.

He also found that guinea-pigs which had been sensitized with serum did not react to milk.

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Lewis [88] considers that the incubation period of the hypersensitive reaction is not sharply limited, but that there is a progressive increase in sensitiveness from the sixth day, possibly before that, extending over a period of several weeks.

He confirmed our results as to the transmission of the hypersensitive reaction from mother to young, but found that all of the young of sensitive mothers were not equally sensitive. He considered that the anaphylactic state depended on the development of a special antibody during the incubation period, which may be transferred passively to an untreated animal. There is also in the serum of hypersensitive guinea-pigs an uneliminated horse serum element or "rest" which is distinct from the antibody. This antibody may be entirely neutralized by the gradual introduction in twenty-four hours of increasing doses of serum.

Lewis,[89] in his second paper, reports studies chiefly on the hypersusceptibility of young guinea-pigs, born of treated mothers. He found that when the defibrinated blood of such

young guinea-pigs was injected into untreated guinea-pigs, they were rendered hypersensitive to an injection of serum within a few hours, but if the injection of horse serum was delayed until after the incubation period, they failed to react. He discusses in some detail the mechanism of anaphylaxis, holding to the view that the sensitizing injection results in the formation of an antibody.

He also reports some work with a serum which had caused severe symptoms in a man about thirty minutes after injection. He did not find this serum materially different in toxicity from the various normal serums when tested on sensitive guinea-pigs.

By purifying horse serum with ether and then precipitating with ammonium sulphate, Gay and Southard [57] found that, after several precipitations, the last fraction was as highly toxic as horse serum, but distinctly less sensitizing than either whole horse serum or the first fraction. The first fraction, obtained by one-third saturation with ammonium sulphate (euglobulins), is as highly sensitizing as whole horse serum; it is analogous to anaphylactin and apparently a purely sensitizing substance without admixture of the toxic elements of horse serum. This euglobulin is absolutely non-toxic for sensitive animals. Repeated large doses not only cause no refractory phase but shorten the period of incubation. It sensitizes normal animals in a few days (four or five).

Gay and Southard [59] state that increased susceptibility in sensitized animals is due to the continued presence in the circulation of an unneutralized element of horse serum (anaphylactin), which acts as an irritant or stimulant to the body cells and in some way causes them to assimilate over-rapidly certain other elements of horse serum. They believe that anaphylactin is not an antibody to horse serum, but some retained substance. They also express the belief that the intoxication of animals by horse serum is not due to a toxic substance formed of antibodies plus antigen and that moreover the reaction of intoxication takes place, not in the circulation of the animal, but in the prepared cell.

Gay and Southard [60] emphasize the point that a sensitive

animal intoxicated with a large dose of serum and recovering passes thereby into a refractory state (antianaphylaxis), and by the same mechanism again becomes sensitive to the toxic effects of horse serum.

Gay and Southard [61] state that animals sensitized with either egg-white or milk will react more or less characteristically to horse serum. After sensitization with egg-white guinea-pigs will react faintly to milk; after sensitization to milk they will react slightly to egg-white. They conclude from this that anaphylaxis is only relatively specific.

Gay and Southard [62] found macroscopic hemorrhages in one or more organs in 85 per cent. of the guinea-pigs which either died of a second dose of horse serum or were killed within twenty-four hours after the second injection. The cause of death, when it occurs, is respiratory. Respiration ceases in the inspiratory phase and shows itself anatomically and histologically as emphysema. The most striking functional feature is severe diaphragmatic spasm. The application of horse serum to the exposed vagus of a sensitive guinea-pig produces severe respiratory symptoms. Gay and Southard regard the changes in the respiratory centres as a physical rather than as a chemical nature. They state that neither hemorrhage nor respiratory death is an indispensable feature of this disease. All guinea-pigs so far examined show fatty changes in many regions involving single muscle fibres or other cells.

Rosenau and Anderson [6 136] report the results of further studies on anaphylaxis. It is shown that the period of incubation is about seven days in guinea-pigs sensitized in the brain and about nine days in guinea-pigs sensitized subcutaneously. It also appears that the sensitization comes on somewhat gradually. The period of incubation is quite constant and is not appreciably prolonged by a large sensitizing dose. Sensitized guinea-pigs probably remain sensitive throughout the rest of their lives, at least 732 days.

The effect of heat and chemical substances is again studied in relation to the sensitizing and toxic principles in horse

serum. The specific nature of anaphylaxis is further shown by various experiments. Three separate anaphylactins have been demonstrated in the blood of a guinea-pig. It is shown that the substance known as anaphylactin is not present during the period of incubation; and may be demonstrated in the blood serum of immune guinea-pigs. Congestion and hemorrhages are sometimes found in guinea-pigs dead of anaphylaxis. Fatty lesions were not discovered. These morphologic alterations, it is believed, do not explain the mechanism of anaphylaxis.

It is shown that horse serum used in cases followed by sudden death in man is no more toxic for guinea-pigs than antitoxic horse serum used extensively in human therapy without untoward symptoms. The belief is expressed that it is not the special toxicity of the horse serum, but the sensitization of the patient, which accounts for the collapse or sudden death sometimes following the injection of horse serum. The essential lesion in serum anaphylaxis is probably localized in the respiratory centre, and the association of asthma with hypersusceptibility to horse serum must be considered in the use of antitoxin. A relation between the toxæmias of pregnancy and anaphylaxis is suggested.

It is shown by Rosenau and Anderson [135] that guinea-pigs cannot be sensitized with guinea-pig fetal blood. Female guinea-pigs, however, may be sensitized with placental extract. After an interval of twenty-two days or longer they show symptoms of protein anaphylaxis when given a second injection of guinea-pig placental extract. It therefore seems that the mother guinea-pig may be sensitized with the autolytic products of her own placenta. These experiments suggest that there may be a certain relation between some cases of puerperal eclampsia and the phenomenon in the guinea-pig.

Besredka [19] confirmed the results reported by Rosenau and Anderson, that guinea-pigs are easily sensitized to a second injection of milk. He found that they responded markedly to a second injection when given into the brain, and that guinea-pigs which had been injected with other substances than milk at

the first injection, supported without ill effects an intracerebral injection of 0.25 c.c. of milk, thus showing the specificity of the reaction.

He found that when milk had undergone the lactic acid fermentation, the curd contained the sensitizing, as well as the toxic properties of the milk, while the whey (*petit lait*), even when given into the brain, failed to cause symptoms in a sensitive guinea-pig.

Besredka [21] refers to his preliminary note on the same subject in which he brings forth his ideas as to the presence in normal serum of the two substances, *sensibilisinogène* and *antisensibilisine* and the properties and action of the two.

In his later article, he gives the details of his experiments on which he bases his conclusions. He believes, as stated in his first paper on the same subject, that it is the combination of the *sensibilisine* and *antisensibilisine* in the nucleus of the nerve cells that produce the anaphylactic shock. This shock may be lessened either by large doses given during the period of incubation, or by small repeated doses after the period of incubation, so that this union of the *sensibilisine* and *antisensibilisine* takes place more slowly.

The shock may also be lessened by renderng the nerve cells indifferent to this brusque union, as by ether narcosis. Anti-anaphylaxis is due to a desensitization, followed by a return of the guinea-pig to a normal state. If the antianaphylaxis is not definite, following a large injection of the serum, it is because the surplus of *sensibilisinogène*, which remains after the elimination of the *antisensibilisine*, is capable of resensitizing the animal.

Wells [177] suggests that the toxic effect of a second dose of protein injected into an animal that has been sensitized by an injection of the same protein two weeks or more previously may be dependent on the aromatic radicals of the protein.

Wells [178] in a most interesting article takes up a study of the chemistry of the substances involved in anaphylaxis and the chemistry of the reaction itself. The major portion of his work was with pure egg-albumen, purified by repeated

crystallization. He found that this pure egg-albumen would sensitize guinea-pigs in doses of one-twenty-millionth of a gramme; fatally, in doses of one-millionth of a gramme. The minimum fatal dose for sensitized guinea-pigs, given intraperitoneally, was about 0.5 milligramme, and about 0.1 to 0.05 milligramme when injected into the circulation. As so small a quantity, 0.1 milligramme, of pure egg-albumen is fatal for sensitized guinea-pigs, he thought it improbable that the injected protein itself could cause death by splitting up and liberating poisonous substances in the space of time in which the reaction occurs. He thought that the minuteness of the minimum sensitizing and intoxicating doses of pure egg-albumen seemed to indicate conclusively that, at least with this protein, both the sensitizing and intoxicating agents were one and the same kind of protein molecule, or else two different proteins of the same molecule.

He found that milk did not lose its sensitizing and intoxicating power when heated to 100° for thirty minutes, and inclined to the belief that this might be due to the fact that the caseinogen of milk is not coagulated by boiling.

Iodization of serum and pure egg-albumen did not alter the specificity of the reaction when these substances were used on guinea-pigs.

Nicolle, Nicolle and Pozerski and Nicolle and Abt published three articles early in 1908 which may be reviewed together.[102] We cannot here follow these authors fully in detail and must satisfy ourselves with citing the principal points. The facts of protein hypersusceptibility are explained as follows:

The substance injected contains a toxic element which is set free by specific antibodies in the hypersusceptible animal. Hypersusceptibility may be produced by developing this antibody, to which the authors give the general name of lysin; thus, toxolysin, albuminolysin and cytolysin. They assume that a coagulation caused by another antibody is necessary. This antibody is called the coagulin; thus, toxocoagulin, albuminocoagulin, cytocoagulin. All the antigens produce anti-

bodies at the same time. Depending on their respective quantities, there is either hypersusceptibility or immunity. The order of events following a reaction would be: First, the fixation of the antibody by the antigen, then the coagulation of the antigen, then the slow and silent destruction by the lysin.

These antibodies have not yet been demonstrated *in vivo* or *in vitro;* but the authors state that there is no reason why they may not exist as precipitins, acting only *in vitro.*

Hypersusceptibility occurs when there is a rapid lysis of a sufficient quantity of antigen. On the other hand, immunity manifests itself when there is first coagulation of the antigen. The lysis then follows slowly, liberating a little of the poison at a time. The two phenomena may coexist.

Otto [106] gives a comprehensive review of the work on anaphylaxis and the serum disease to date. He discusses the historical development of the subject,—the serum disease in man, experimental anaphylaxis especially in guinea-pigs, and concludes with an admirable review of the theories which have thus far been brought forward to explain hypersusceptibility. The bibliography contains seventy references to the literature on the subject.

Richet,[124] in studying the anaphylactic properties of actino-congestine, suggests as a rational theory that the injection of the actino-congestine causes at the end of about two weeks (period of incubation) the formation of a new substance, which he calls *toxogénine,* which alone is harmless, but in the presence of the original poison becomes hypertoxic.

In this article he gives some interesting details of experiments on dogs, in which, by injecting untreated dogs with the serum of treated dogs, he was able to render the untreated animals very susceptible to a first injection of actino-congestine.

Hamman [72] states that the views of Wolff-Eisner seem to fit best into what we at present know of the tuberculin reaction and, shorn of their technicality, are briefly this: Tuberculin,

which really consists of ultramicroscopic portions of the tubercle bacillus, produces the same effects in the animal body as the tubercle bacilli, except that the latter are capable of multiplication. The local tuberculin reaction is caused by an accumulation of lymphoid cells, and true giant cells are formed. The injection of living tubercle bacilli is followed by the development of hypersensitiveness the same as that which follows tuberculin injections. As in typhoid and cholera, the immunity reaction in tuberculosis depends on the presence of lysins. The tuberculin reaction is not due to the tuberculin itself nor to the disintegration at the site of disease, but to a new toxic substance formed by the action of the lysins on the albuminous portion of the bacillary body. For this toxic substance to become active the organism must be in a condition of hypersensitiveness. There are, then, two factors to be considered: the presence of lysins and the condition of hypersusceptibility. The lysins may be artificially increased and are the bodies which deviate complement in Wassermann's experiment. The hypersensitiveness varies under conditions of which we are still ignorant, but it, too, may to some extent be stimulated or blunted. Normally there is no lysin present, or so small an amount that when tuberculin is injected the transformation goes on so slowly that the newly formed toxin causes no appreciable effect. Under continuous minimal stimulation the quantity of lysin increases, and the transformation then occurs so rapidly that after injection intense toxic symptoms occur. The toxin causes a local reaction at the site of injection, constitutional symptoms and an inflammatory reaction at the site of the tuberculous lesion. During the inoculation of small doses of tuberculin hypersensitiveness is developed. Variation in susceptibility to tuberculin in tuberculous subjects depends essentially on variation in hypersensitiveness, lysins probably being present in all.

Weil-Halle and Lemaire[176] found that if untreated guinea-pigs were injected at the same time with 0.01 c.c. of horse serum and 4 c.c. of the serum of a rabbit, which had previously been

given horse serum, many of the guinea-pigs died in from eight to fifteen days. If the rabbit was bled in less than ten days or after sixty days, no ill effect was caused by the injections.

Remlinger [115] having noticed that the injection of one large dose or several repeated small doses of the nervous tissue of the brain of different species produced in certain animals emaciation, cachexia and even death, endeavored to determine if this was due to an anaphylactic action of the nerve substance.

He injected dogs at intervals of ten to fifteen days subcutaneously with nerve tissue from the rabbit; guinea-pigs, with rabbit nerve tissue, and rabbits, with either rabbit, guinea-pig or dog nerve substance. After a variable number of injections, they were all tested by the intracerebral injections of the nerve substance of the same species used in the preliminary tratment. He never noticed the least morbid phenomenon.

These results are open to the criticism that the use of several injections, especially in the guinea-pig, may have immunized the animals to the test injection.

Grunbaum [65] reports some observations in eleven cases in which antituberculosis serum had been administered. In no case did the second injection cause any uncomfortable effect; the fourth injection, in one case, was followed by an urticarial eruption; in another case, œdema of the tongue and larynx followed two successive injections, and in a man thirty-one years of age, the twentieth injection was followed almost at once by cyanosis, vomiting, collapse and death in five minutes.

Grunbaum attributes these accidents to an individual susceptibility.

Banzhaf and Famulener [12] found that anaphylactic symptoms could not be prevented by ether narcosis, morphine sulphate or calcium chloride. They found that if chloral hydrate was used to produce hypnosis in sensitive guinea-pigs, about 75 per cent. were completely protected against the injection intraperitoneally of 5 c.c. of serum, while 90 per cent. of the controls died.

Pozerski [110] found that repeated injection of papain into guinea-pigs, very much less than the minimum lethal dose, at intervals of four or five days, produced after the third, fourth or fifth injection an undoubted state of anaphylaxis with death, accompanied by the same post-mortem appearances as are found in death from a single large dose.

Moro [58] assumes that the percutaneous reaction is a late reflex; that is, an angioneurotic inflammation. This view is confirmed by the fact that the reaction occurs at other points of the body than where the tuberculin lanolin is applied. The cutaneous reaction is often symmetric and suggests, according to Moro, hypersusceptibility of the sympathetic nervous system.

Gillette [43] reports sudden death in an asthmatic following the injection, five minutes previously, of 2000 units of diphtheria antitoxin. The postmortem failed to shed any light on the case.

SYMPTOMS CAUSED BY THE INJECTION OF HORSE SERUM INTO A SENSITIZED GUINEA-PIG.

Very characteristic symptoms are produced by horse serum, either normal or antitoxic, when injected into a susceptible guinea-pig. The symptoms are apparently the same whether the injection is made subcutaneously or into the peritoneum, or whether normal or antitoxic serum is used. In five or ten minutes after injection the pig becomes restless and agitated; then manifests indications of peripheral irritation or respiratory embarrassment by scratching at the mouth, coughing, and sometimes by spasmodic, rapid or irregular breathing. This stage of exhilaration is soon followed by one of paresis or complete paralysis. The pig is unable to stand, or, if it attempts to move, falls on its side; when taken up it is limp. Spasmodic, jerky, and convulsive movements now supervene.

Pigs in this state with complete paralysis may fully recover; but usually convulsions appear, and are almost invariably forerunners of death. Symptoms appear in about ten minutes

after the injection has been given; occasionally in pigs not very susceptible they are delayed thirty to forty-five minutes. Only in one or two instances of the many hundred pigs which we have observed have the symptoms developed after one hour. Pigs developing symptoms as late as this are not very susceptible and do not die. The chain of symptoms is exceedingly characteristic. The symptoms do not always follow in the order given. Death usually occurs within an hour and frequently in less than thirty minutes.

If the second injection be made directly into the brain the symptoms are manifested with explosive violence, the animal frequently dying within two or three minutes. The same is also true if the second injection be made directly into the circulation.

We took the temperature of a number of guinea-pigs twice daily for eighteen days following the injection of large quantities of horse serum subcutaneously, in order to determine whether a febrile reaction followed. No marked deviation from the normal temperature was noted.

Normal horse serum, when injected into normal guinea-pigs, causes no symptoms. Large amounts, such as 6 or 10 c.c., may be injected into the peritoneal cavity of a guinea-pig without any apparent inconvenience to the animal. When normal horse serum is injected subcutaneously into the guinea-pig it is sometimes either absorbed very slowly or there is a slight local reaction, as indicated by œdema and induration of the subcutaneous tissue at the site of inoculation.

THE POISON ACTS ON THE RESPIRATORY CENTRE.

Judging from the symptoms produced by the injection of horse serum into a susceptible guinea-pig we assumed that the poison acted on the central nervous system. Autopsies done immediately after the death of the guinea-pigs showed invariably that the heart continued to beat after respiration had ceased. In some instances the heart would continue to beat a full hour when exposed. This would seem to indicate that

we were dealing with a poison which caused death through the nervous control of the respiration, and experiments show that this effect is certainly not local.

PERIOD OF INCUBATION.

A certain time must elapse between the first and the second injection of the foreign protein before the toxic action is manifest. This period of incubation is from seven to twelve days and corresponds suggestively with the period of incubation of the serum disease, which Von Pirquet and Schick place at eight to thirteen days, and with the period of incubation of some of the infectious diseases.

If a guinea-pig be given an injection of serum and then be injected again any time during the period of incubation no ill results follow. In other words, the animal has not had time to enter a state of anaphylaxis to the foreign protein. If, however, the injection be given after the seventh to the twelfth day the animal is then in a state of anaphylaxis or hypersusceptibility to the foreign protein. The animal remains susceptible a very long time. The longest period which we have observed is 732 days between the first and the second injection. There is no reason to doubt that guinea-pigs remain susceptible during their entire lives.

We found that the period of incubation appeared about the seventh day in guinea-pigs sensitized in the brain and about the ninth day in guinea-pigs sensitized subcutaneously. So far as may be judged, it therefore appears that the period of incubation is somewhat shorter in guinea-pigs sensitized in the brain than in those sensitized subcutaneously. It also seems quite evident that the sensitization comes on somewhat gradually. Judged by our results and the work of others the period of incubation is quite constant.

Lewis states that the period of incubation is not to be considered as abruptly terminating at a given day. He says that he has made an animal quite sick by the intracardial injection of 2 c.c. of serum on the sixth day after the toxin-antitoxin

mixture. We have obtained suggestive symptoms on the fourth and the fifth day.

So far as may be judged from our work, the period of incubation is not appreciably prolonged by a large sensitizing dose.

THE SENSITIZING SUBSTANCE.

We have suggested that the protein which sensitizes the guinea-pig is the same as that which later poisons it; profound changes are produced by the first injection. These changes localize themselves in the central nerve cells at the second injection. Our subsequent work has evolved nothing to alter this working hypothesis.

Guinea-pigs may be sensitized by administering the foreign protein subcutaneously, intraperitoneally, intracerebrally, directly into the circulation, or by feeding.

We have shown that the filtrate from horse serum, after precipitation with ammonium sulphate, still possesses sensitizing powers in spite of the fact that this filtrate contains but little of the serum globulin and is very weak in antitoxic value.

Formaldehyde does not appear to modify the sensitizing property in horse serum, though it is capable of destroying the toxic properties of tetanus [5] and diphtheria toxins.

From a limited number of experiments it seems that the sensitizing principle is not dialyzable through a collodion sac when placed in the peritoneal cavity of a guinea-pig.

The sensitizing principle is not affected by the various preservatives used for the preservation of antitoxic serum, by drying, by precipitation with ammonium or magnesium sulphate, or by admixture with diphtheria or tetanus toxins.

The removal of the spleen or the thyroid from the animal before or after receiving the sensitizing dose apparently has no effect on the development of anaphylaxis.

We have found that the sensitizing principle of horse serum is gradually influenced by heat and almost entirely disappears when the liquid serum is heated to 100° C. for one hour. Pigs

sensitized with small quantities of horse serum heated to 100° C. for one hour, when subsequently tested, develop very slight symptoms.

Guinea-pigs may readily be sensitized by intracerebral injections, provided quantities of 0.0001 c.c. or more are used. We obtained negative results with sensitizing doses of 0.00001 c.c.

The blood serum or an emulsion of the brain substance of a sensitized guinea-pig, when mixed with horse serum, does not modify the sensitizing power of the horse serum.

Some breeds of guinea-pigs appear to be more susceptible than others. The American guinea-pigs seem to be most susceptible, the German breeds not quite so much, and the French somewhat less, while the guinea-pigs tested by Vasconcellos in Rio de Janeiro scarcely respond at all.

THE TOXIC PRINCIPLE.

At one time we made efforts to isolate the active principle in horse serum which causes the symptoms, but as soon as we realized that the toxic principle present in horse serum exerted its action in quantities so minute as to place it almost in the category with the ferments, we realized how difficult it would be, with the present technic, to isolate this substance. Nevertheless, we devoted much time and study to the relation of this toxic principle to various chemical, physical, and electrical influences. The practical importance of eleminating this toxic principle from horse serum, or of neutralizing it, is at once evident.

The toxic principle is not affected by various chemicals, such as calcium chloride, sodium nitrate, magnesium sulphate, ammonium sulphate and formaldehyde.[5] It is not affected by various ferments, alkaloids and similar substances, such as *takadiastase*, pancreatin, rennin, myrosin, invertin, emulsin, pepsin in acid solution and in alkaline solution, *ingluvin*, malt, atropine, strychnine, morphine and caffeine.

It is not affected by freezing at 15° F., or by filtration

through porcelain, drying, precipitation and dialysis, or exposure to the X-rays.

We found it interesting to compare the toxic effects on sensitive animals of untreated antitoxic serum, and the precipitated refined antitoxin; bulk for bulk we found them equally toxic. But as the same number of units can be given in half the bulk there is a manifest advantage in using the precipitated serum, as the rashes and other untoward effects of serum depend to some extent on the volume of serum administered.

The smallest amount of serum given intraperitoneally that we have found to cause the death of a guinea-pig is 0.1 c.c. One hundredth of a cubic centimetre, when given directly into the heart, is sufficient to cause the death of the animal, while 0.25 c.c. given into the brain is almost invariably fatal. In most of our work, however, we have used 5 or 6 c.c. of serum intraperitoneally, and this seems to be the favorite dose of other workers. Certain symptoms in guinea-pigs caused by a second injection of the serum suggested to us that the action might be due to hæmolysis or the formation of precipitins. By a large number of experiments, however, we were able to exclude both hæmolysis and the formation of precipitins as factors.

Sensitized animals were given various chemicals the day before the second injection of serum. No influence on the anaphylactic state was obtained by these substances. The following were used: pancreatin, potassium oxalate, sodium sulphate, magnesium sulphate, peptone, calcium chloride and calcium acetate.

Obermayer and Pick find that when the aromatic radicals of a protein are combined with various substances the protein loses the power to produce precipitins of closely allied specificity for the original species. Their results suggest that the aromatic groups of the molecule are closely related to the species specificity. This indicates that the striking specificity of proteins of different species depends on the aromatic groups of the protein molecule and Vaughan has found evidence that the toxicity of the proteins depends on these same groups.

Fleischmann also finds that tryptic digestion destroys this characteristic species specificity.

We tested a large number of guinea-pigs to determine this point but found that, so far as the toxicity of horse serum is concerned at the second injection, it was not appreciably modified by iodine.

A few experiments were made to determine the relation of methæmoglobin-producing substances, such as nitrates, on the symptoms. Sensitive guinea-pigs were given subcutaneously an injection of sodium nitrite. In thirty minutes the exposed mucous membranes appeared distinctly blue; they were then tested for their susceptibility to horse serum and found to react in the usual way. Controls showed that the quantity of nitrite used was not sufficient in itself to kill the guinea-pigs.

Besredka reported some interesting observations concerning the prevention of anaphylaxis by ether narcosis. He stated that if sensitive guinea-pigs were etherized to the stage of complete relaxation and while in this state injected intracerebrally with normal horse serum, and the administration of ether continued a short time, the animal continued to sleep after the injection and at the end of about half an hour awoke without presenting the least symptoms of anaphylaxis. If the guinea-pig were tested on the following day it would be found to be immune.

Of eight guinea-pigs on which we tried this experiment with ether, seven died from the effects of the second injection of horse serum. It is our belief that the guinea-pig which recovered had masked symptoms while under the influence of the ether and probably would not have died in any case, for we have a certain number of recoveries from the intracerebral injections of 0.2 c.c. of horse serum. It is true, however, that the narcosis masks the symptoms. The difference in our results may be accounted for either by the difference in toxicity of the French and the American serums, or, more likely, by the difference in susceptibility of the animals used.

Normal horse serum may be heated to 90° C. for one hour and still remain slightly toxic when injected into a sensitized

guinea-pig. Its toxicity, however, is evidently markedly affected. Heating to 70° C. for one hour does not seem to diminish appreciably its poisonous properties, but it appears to be affected at 80° C. for one hour. At 100° C. for one hour the toxicity apparently disappears.

It appears that there is a slight difference between the sensitizing and toxic principles in horse serum so far as the resistance to heat is concerned. Serum heated to 100° C. for one hour retains some power of sensitization, but seems to lose its toxicity when given at the second injection. This difference may be more apparent than real, for exceedingly minute amounts are sufficient to sensitize guinea-pigs, while a very large quantity of weakened serum would be necessary to produce symptoms. It must be remembered that in our experiments 20 c.c. of the dilution represents but 5 c.c. of serum.

These facts must be kept in mind when drawing conclusions from work on split proteins, fractional precipitation, or other methods to isolate the sensitizing substance in pure form. A very minute amount of the original protein substance in horse serum clinging to the globulin, or other substances modified by chemical methods, might be sufficient to sensitize guinea-pigs, whereas it would require very large amounts of such a modified protein to poison a sensitive animal.

THE SPECIFIC NATURE OF ANAPHYLAXIS.

From our first studies upon hypersusceptibility we were interested in the question whether this reaction was specific.

In our first work on this subject we showed that this reaction was quantitatively specific for serums. That is, guinea-pigs sensitized with horse serum are subsequently very susceptible to a second injection of horse serum, but only slightly if at all susceptible to a second injection of the serum of other animals, such as rabbit, cat, dog, hog, sheep, chicken, or man. Conversely, guinea-pigs sensitized with the serum of these other animals are not very sensitive to a second injection of horse serum, whereas they respond actively to a subsequent

injection of the same kind of serum as that used for the first injection.

We have further shown that the specific nature of this phenomenon is more marked when protein substances of widely different nature are used at the first and second injections. Thus, a guinea-pig sensitized with horse serum does not react at all to a subsequent injection of egg-white, vegetable protein, or milk. A guinea-pig sensitized with milk does not react to the other protein substances mentioned, etc.

We have succeeded in demonstrating more clearly the specific character of the phenomenon we are studying by proving that guinea-pigs may be in a condition of anaphylaxis to three protein substances at the same time. For instance, a guinea-pig may be sensitized with egg-white, milk, and horse serum, and may subsequently react to a second injection of each of these substances within a brief period of time. The guinea-pig may be sensitized by injecting these strange proteins either at the same time or at different times, in the same place or in different places, or by injecting them separately or mixed. The guinea-pig differentiates each anaphylactic-producing protein in a perfectly distinct and separate manner. The animal is susceptible to the second injection of each one of the three substances in the same sense that it is susceptible to three separate diseases. These distinct reactions, in so brief a time, seem to accentuate the specific nature of the phenomenon we are studying. It also adds weight to our belief that profound chemical changes, probably in the central nervous system, rather than morphologic alterations, explain the essential features of the reaction.

Further evidence on the specific nature of the phenomenon will be found in the discussion of anaphylactin.

ANAPHYLACTIN.

Gay and Southard first discovered the interesting fact that if the blood of a guinea-pig which has received a small sensitizing dose of normal horse serum be drawn, the serum collected, and 1.5 c.c. of this given to a normal guinea-pig, the

animal is rendered susceptible to a subsequent injection of horse serum fifteen days later.

Otto then showed that the animals react if tested within twenty-four hours.

Gay and Southard applied the name "anaphylactin" to this substance in the blood of sensitized guinea-pigs. They considered this substance a portion or "rest" of horse serum.

The sensitizing substance or anaphylactin (called *sensibilisinogène* by Besredka) is present in the blood serum of a sensitive animal. We have limited the word anaphylactin to indicate that substance in the blood serum of a sensitive animal which, when transferred into a normal animal, is capable of sensitizing it within forty-eight hours. It must be present in an exceedingly minute amount, for we have shown that the blood of guinea-pigs receiving only 0.002 c.c. of serum contains this substance several months later, and 1.5 of the serum of such animal, when injected into a normal animal, renders it sensitive to a subsequent injection of horse serum twenty-four hours later.

It is of some interest to determine just when anaphylactin appears in the blood of a sensitized guinea-pig, particularly whether its presence may be demonstrated during the period of incubation. We found no indication of this substance in the blood of sensitized guinea-pigs until the ninth or tenth day, *i.e.*, just about the time necessary to render guinea-pigs sensitive.

We have found that anaphylactin is present in the blood serum of immune guinea-pigs. This fact is of importance in the consideration of the mechanism of anaphylaxis. From this we may argue that a true condition of immunity exists, for the sensitizing substance is certainly present in the flowing blood, but the organism as a whole or its susceptible cells are protected by a neutralizing antibody.

We have referred above to the fact that guinea-pigs may be in a condition of anaphylaxis to three protein substances at the same time. We later found that the substance in the blood serum of sensitized guinea-pigs known as anaphylactin is also

specific in the same sense. By transfusing the blood serum of guinea-pigs sensitized to horse serum, egg-white, and milk, three separate and distinct reactions were obtained in the guinea-pig into which this serum was transferred.

LESIONS.

Gay and Southard, 1907, found lesions in guinea-pigs dying from a second injection of serum, and in those which had severe symptoms and were later chloroformed, which they interpreted as explaining the mechanism of anaphylaxis. They state that, "the study of the histopathology of this serum disease shows us that we have to deal with an intimate cell reaction, demonstrable by definite cell lesions." These investigators state that considerable hemorrhages, rather definitely localized, are the characteristic gross lesions. The hemorrhages may be in one or several organs, gastic hemorrhages being especially frequent. Microscopically, there are, in addition to the naked-eye hemorrhages, minute interstitial and oozing hemorrhages. They also found fatty changes in voluntary muscle fibre, heart muscle fibre, and nerve fibre.

That the congestion and dilatation of the blood-vessels found in the abdominal cavity and the hemorrhages on the mucosa of the stomach are not characteristic of death due to anaphylaxis is evident from the fact that we have found that in violent death produced by large subcutaneous injections of chloral cyanhydrin or hydrocyanic acid there are somewhat similar congestions and hemorrhages. Further, we have lately had the opportunity to examine a guinea-pig whose death was caused by suffocation in an atmosphere of carbon dioxide. In the stomach and lungs of this guinea-pig lesions were found that, so far as the congestion and hemorrhages are concerned, were similar to those described in guinea-pigs dying from a second injection of horse serum.

We were especially struck by the fact that macroscopic congestions and hemorrhages were frequently absent in guinea-pigs poisoned by a second injection of horse serum given into the brain.

Finally, this congestion and dilatation of the vessels of the abdominal cavity is well known to occur in shock and other states.

We were unable to confirm Gay and Southard's findings in regard to the fatty changes.

It is noticeable that one of the conspicuous lesions of the serum disease and other reactions to foreign proteins consists of an angioneurotic œdema. In serum anaphylaxis as seen in the guinea-pig the irritation of the skin and mucous membranes of the mouth may be of the nature of an angioneurotic œdema. One might imagine a localized œdema of this character in the ganglia about the respiratory centres to account for the serious symptoms or death.

FEEDING EXPERIMENTS.

Guinea-pigs may be sensitized by feeding them meat or serum.

We found that guinea-pigs could be sensitized by feeding them for some days on horse meat, or dried horse serum, mixed with their food. We did not use a stomach-tube, as the possibility of making slight wounds in the œsophageal or gastric mucosa would vitiate the feeding experiments, as we know from our previous work that very small quantities could readily sensitize the animal to a subsequent injection of serum.

The guinea-pigs that had been fed with horse meat or horse serum, after an interval of at least four days, were injected with horse serum and reacted in a characteristic manner.

We also found that guinea-pigs could be sensitized to cattle serum by feeding them with beef. Cooking the meat entirely destroyed its sensitizing properties.

The fact that guinea-pigs may be rendered susceptible by the feeding of strange protein matter opens an interesting question as to whether sensitive guinea-pigs may also be poisoned by feeding with the same serum given after a proper interval of time. If man can be sensitized in a similar way by the eating of certain protein substances, may not this throw

light on those interesting and obscure cases in which the eating of fish, sea food, and other articles of diet habitually cause sudden and sometimes serious symptoms?

MATERNAL TRANSMISSION.

In the course of our work we had the opportunity to test the susceptibility of the young of susceptible guinea-pigs and we found that hypersusceptibility to the toxic action of horse serum is transmitted from the mother guinea-pig to her young. This function is solely maternal; the male takes no part whatever in the transmission of these acquired properties. Whether this maternal transmission is hereditary or congenital cannot be definitely stated.

We were able to exclude the milk as a factor in transmitting the hypersusceptibility to the toxic action of horse serum by a series of "exchange" experiments. Exchange experiments consist in at once placing guinea-pigs born of a susceptible mother to nurse with an untreated female while, in exchange, the young of the untreated female are placed to nurse with the susceptible female. From these exchange experiments we learn that the hypersusceptibility is not transmitted to the young in the milk.

We also learned from our experiments that hypersusceptibility may be transmitted from mother to young, whether the mother is sensitized before or after conception.

If an anaphylactic tendency be transmitted from mother to young in man it may explain the severe reaction and death that occasionally take place following a first injection of serum.

These results on the hereditary transmission of the susceptibility to the poisonous action of horse serum in guinea-pigs may throw light on the well-known inherited tendency to tuberculosis in children born of a tuberculous parent.

There are certain analogies between the action of tuberculosis and horse serum. Both produce hypersensitiveness and also a certain degree of immunity. Now that we have proved that this hypersensitiveness or anaphylactic action in the case of horse serum may be transmitted hereditarily in guinea-pigs,

may it not throw light upon the fact that tuberculosis "runs in families"? While there are several recorded instances demonstrating that immunity to certain infectious diseases may be transmitted from a mother to her young, this is, so far as we know, the first recorded instance in which hypersensitiveness, or a tendency to a disease, has been experimentally shown to be transmitted from a mother to her young.

IMMUNITY TO ANAPHYLAXIS.

We showed in our first publication on the subject of anaphylaxis that guinea-pigs may be actively immunized against this phenomenon. At the same time we demonstrated that the immunity could not be transferred passively to other animals in the blood or body juices.

Guinea-pigs may be actively immunized in several ways: (1) by repeated injections of serum during the period of incubation, *i.e.*, during the first ten days, before the animal reaches the state of hypersusceptibility; (2) animals that recover from a second injection given during the anaphylactic stage are at once immune.

The fact that guinea-pigs cannot be immunized passively by the transfer of blood or body juices would make it appear that the "immune body," if such exists against the toxic action of horse serum, is not free in the blood or body juices as is the case in diphtheria. In fact, it has been questioned whether the active immunity which we have described is an instance of true immunity, or a "refractory" condition, or even an actual return to the normal.

Subsequent researches have strengthened our belief that we are dealing with a true condition of immunity and not a prolongation of the period of incubation or a return to the normal. Thus, it has been shown that guinea-pigs in the "refractory" condition still contain anaphylactin in their blood. It is at once evident that they have not returned to their normal condition. Further, we have demonstrated that such "refractory" female guinea-pigs will transmit this anaphylactic substance to their young. Only the sensitizing sub-

stance passes into the blood of the fœtus, which is therefore in a condition of hypersensibility. The "immunizing substance" or "condition" is not transmitted.

It seems to us that we have here a striking analogy to that phase or kind of immunity which Von Pirquet describes as "allergie." In other words, we have an acquired immunity associated with anaphylaxis. In guinea-pigs this immunity may follow one attack of the disease, *i.e.*, the serum reaction. As stated by Von Pirquet, "allergie" manifests itself by an immediate reaction and corresponds to the condition of immunity conferred by an attack of smallpox or some of the other acute infectious processes.

In the case of syphilis we have a striking instance in which the virus is not autoinoculable. In the serum reaction in the guinea-pig an analogous train of events occurs, for after the sensitized guinea-pig has responded the reaction renders the organism immune.

ACTION OF HORSE SERUM ON MAN AND OTHER ANIMALS.

It may be that man can not be sensitized in the same way that, as we have shown, guinea-pigs can. We made no human experiments, but experimental data are recorded by others which have a direct bearing on this question.

Repeated injections of horse serum into man are not an infrequent occurrence. Patients suffering from diphtheria are often given injections of antitoxic serum at short and frequent intervals. It is also not rare for persons to have several attacks of diphtheria at long intervals and to be treated each time with antidiphtheria serum.

Certain serums, for example the antitubercle serum of Maragliano or the antirheumatic serum of Menzer, are habitually used by injections at intervals of days or weeks.

In all these cases of frequent or repeated injections the amount which has been injected and the interval between the injections must be taken into account in relation to this work. Von Pirquet and Schick, in their work on *Serumkrankheit*, give eight instances in which children received two injections

of horse serum at intervals of sixteen to forty-two days between the first and the second injections. All these eight cases show this in common, that after the first injection of horse serum the symptoms of the serum disease appear after the normal period of incubation, *i.e.*, between the eighth and the thirteenth days. But when the individuals are again injected with horse serum after intervals of sixteen to forty-two days symptoms of the serum disease reappear at once or at least within twenty-four hours.

Von Pirquet and Schick give a list of 60 children who were injected with antitoxic horse serum at intervals of six days to seven and a half years between the first and the second injections. They found that when the second injection was given from fourteen days to four months after the first injection they obtained, with great regularity, what they termed the "immediate reaction"; but when the interval between the first and second injections was over four months they obtained little or no immediate reaction but what they termed "an accelerated reaction," for the fever, urticaria and other symptoms of the disease appeared on the fifth, sixth, seventh, or eighth day. It will be remembered that the normal period of incubation for the symptoms of the serum disease to appear after the first injection is between the eighth and the thirteenth days. Von Pirquet and Schick lay special stress on the phenomenon of the "immediate" and the "accelerated" reactions following the second injection.

We might also conclude, despite the suggestion in our work on sensitizing guinea-pigs by feeding them with horse serum or horse meat, that children are not sensitized to the toxic action in horse serum by eating horse meat, from the fact that horse meat is a favorite article of diet in certain European countries, and that there is nothing on record to show that the injection of horse serum in those countries is fraught with more danger than where this practice does not obtain. We must, however, remember that our work has shown that guinea-pigs are sensitized with exceedingly minute quantities of the strange protein, and that repeated injections cause an immu-

nity; and it is possible that the same may be true of feeding. Further, we have shown that cooking destroys the sensitizing property of meat.

Man reacts to the first injection of horse serum after a period of incubation of eight to thirteen days. Guinea-pigs show practically no reaction following the first injection. Both react to a second injection. The reactions in man and the guinea-pig, however, differ both in severity and in kind. The relation, therefore, that our observations on the guinea-pig may have in its application to man must await further study. Of course, the fact that other animals beside man and guinea-pigs react to a second injection of horse serum would seem to indicate that we are dealing with one and the same reaction.

It has been shown that rabbits react after repeated injections, which has also been noted in the use of repeated injections of the serum in man.

THE RELATION OF SERUM ANAPHYLAXIS IN THE GUINEA-PIG TO SERUM THERAPY.

Besredka and Steinhardt were the first to point out that the second injection may be given into the brain of guinea-pigs. When a small quantity of horse serum is injected into the brain of a sensitized guinea-pig the symptoms appear promptly and often with great violence, and death is a common result.

Besredka believes that intracerebral injections may be used as a measure of the toxicity of therapeutic serums. He states that, measured in this way, different serums show a wide gamut of toxicity, the fatal dose varying from $^1/_4$ to $^1/_{128}$ of a cubic centimetre. He believes that this toxicity resides in the serum and not in the cellular elements; further, that the serum of horses living under apparently the same conditions has about the same toxicity, individual variations being rare and of little importance. He concludes that, in a general way, all serums which incite in guinea-pigs grave anaphylactic phenomena in doses of 0.0625 to 0.05 c.c. and *a priori* above this amount should be considered toxic.

We doubt whether there is a relation between the toxicity of serums as tested on guinea-pigs in this way and their power to produce the serum disease or collapse or sudden death in man. It appears to us that in man the symptoms of the serum disease depend partly on the kind of serum and the amount used. The unfortunate accidents, such as collapse and sudden death, depend more on the sensitization of the individual than on the so-called toxicity of the serum used.

Fortunately, we were able to obtain two antidiphtheria serums which had been used in two cases followed by sudden death:

Case I.—Serum No. 2277. Reported by Dr. S. N. Wiley, Norristown, Pa. (*Jour. Am. Med. Assoc.*, 1908, i, 137). Mr. E. W., aged 34 years, splendid physique, best of health. Prophylactic injection of 1000 units antidiphtheric serum. Site of inoculation four inches above Poupart's ligament. Within two minutes had violent symptoms—anxious expression, itching, burning, labored breathing; lips, face and neck swollen and red; paralysis; convulsions. Died within five minutes of injection.

Case II.—Serum No. 2295. Reported by Dr. H. F. Gillette, Cuba, N. Y. (*Jour. Am. Med. Assoc.*, 1908, i, 40). Mr. B., 52 years old. Had asthma and bronchial catarrh. Urine and heart normal. Rheumatic attack fifteen years ago. Coughed and raised plenty of sputum. Injection of 2000 units antitoxic serum under left scapula. Prickling sensation in neck and chest, labored breathing, pulse regular and full. Seized with tonic spasm. Died within five minutes after injection.

From our experiments it is plain that the serums which do not produce untoward symptoms when injected into man are quite as toxic on sensitized guinea-pigs as the serums which have been followed by serious symptoms when injected into man. We believe that the difference lies in the susceptibility of the individual and not in the toxicity of the serum.

It has interested us very much to find that the above two cases, and also others that have come to our notice, were in asthmatics. In our first publication we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory centre, and the association of asthma and hypersusceptibility to horse serum in man would seem to lend weight

to this hypothesis. The knowledge of the fact that the injection of horse serum into some asthmatics is attended with danger must be considered in the use of antitoxin.

HYPERSUSCEPTIBILITY AND IMMUNITY PRODUCED BY BACTERIAL PROTEINS.

We believe that the problem of hypersusceptibility has an important bearing on the question of immunity and hence we have expressed the opinion that "resistance to disease may largely be gained through a process of hypersusceptibility. Whether this increased susceptibility is an essential element or only one stage in the process of resistance to disease must now engage our attention." We cannot escape the conviction that this phenomenon of hypersusceptibility has an important bearing on the prevention and cure of certain infectious processes. Our work on the hypersusceptibility produced by the bacterial proteins strengthens this belief, for our recent results prove that the phenomenon of hypersusceptibility to certain proteid substances extracted from the bacterial cell is followed by a definite immunity against infection by the corresponding micro-organism.

Experimental study of the bacterial proteins is of the greatest importance on account of the practical uses to which results along this line may lead. The relation of these studies to the so-called endotoxins is evident.

Hypersusceptibility may easily be induced in guinea-pigs with protein extracts obtained from the bacterial cell. The first injection of most of the extracts used by us seems comparatively harmless to the animal. A second injection of the same extract shows, however, that profound physiologic changes have taken place. A definite period must elapse between the first and the second injections. The symptoms presented by the guinea-pigs as a result of the second injection resemble those caused by horse serum. The phenomenon induced by a second injection is followed (in certain cases) by an immunity to the corresponding infection.

These results strengthen our belief that the phenomenon of hypersusceptibility has a practical significance in the prevention and cure of certain infectious processes. It also gives a possible explanation of the period of incubation of some of the communicable diseases. Is it a coincidence that the period of incubation of a number of infectious diseases is about ten to fourteen days, which corresponds significantly with the time required to sensitize animals with a strange protein?

In certain infectious diseases with short periods of incubation, such as pneumonia, the crisis which commonly appears about the tenth day may find a somewhat similar explanation. It is evident that disease processes produced by soluble toxins, such as diphtheria and tetanus, do not belong to the category now under consideration.

The hypersusceptibility produced by the colon and typhoid bacilli was followed by a definite immunity to the corresponding infection. In case of anthrax, however, immunity does not follow hypersusceptibility to the anthrax protein. We are not dealing, therefore, with a general law applicable to all infections, but with certain limitations, as in the case of antitoxic immunity.

THE RELATION OF ANAPHYLAXIS TO THE TOXÆMIAS OF PREGNANCY.

The symptoms of puerperal eclampsia and the conditions under which it occurs suggest that anaphylaxis may explain some of the mystery of this state.

It occurred to us that either the blood or protein substance in solution from the fœtus or the placenta may first sensitize the mother. A subsequent introduction into the system of the mother of a similar substance may explain the convulsions and the symptoms which occur in a certain class of the toxæmias of pregnancy.

There seems to be a fair agreement that the placenta must be the source of the toxic material, especially as typical cases of eclampsia and pernicious vomiting have been observed in

patients with hydatid mole, in which cases, of course, toxic matter of fetal origin could be eliminated. Besides, eclampsia may appear after the fœtus has been removed. Much attention was therefore given to the hypothesis elaborated about four years ago by Veit, Weichardt and others that, through the entrance of placental cells into the circulation of the mother, an intoxication was caused either by disintegration of the cells and the formation of toxic substances or by the development of antisubstances in the maternal organism.

In spite of much experimentation and discussion, however, no satisfactory conclusions have yet been reached concerning the validity of this hypothesis, and Martin has secured some very valuable evidence that, at least in rabbits, entrance of their own placental elements into the circulation in large amounts does not cause any serious disturbance.

Along these lines we made a number of experiments to determine whether the fetal blood of the guinea-pig could sensitize the mother guinea-pig. We injected a number of female guinea-pigs, both pregnant and not pregnant, with fetal blood, and, after an appropriate interval, gave them a second injection of the same material. All these experiments resulted negatively, which was anticipated from our previous studies upon the effect of homologous blood serums. This is in harmony with the clinical observations that the poisons causing the toxæmias of pregnancy do not come from the fœtus.

We made a series of experiments on female guinea-pigs with guinea-pig placental extracts. The placenta (almost at full term) was ground up and allowed to autolyze about an hour at room temperature and some of the resulting extract was injected subcutaneously into female guinea-pigs.

From our studies it was evident that the mother guinea-pig may be sensitized with the autolytic products of her own placenta. These experiments naturally suggest that there may be a certain relation between some cases of puerperal eclampsia and the phenomenon in the guinea-pig which we are studying. Further studies along this line are now being made.

AN EXPLANATION OF THE PERIOD OF INCUBATION OF CERTAIN INFECTIONS.

The only plausible explanation of the period of incubation of an infectious disease heretofore considered has been that it required a certain time for the infective principle to grow in sufficient amount in the body to produce symptoms. This view is not tenable in many cases. The period of incubation in many cases is independent of the amount of infection.

In view of the studies on anaphylaxis we now have a more likely explanation of the period of incubation in certain cases. If the body is sensitized by the foreign protein dissolved out of the infecting organism it would require a certain definite time before the poisonous effects are felt. This not only explains why a certain time must elapse between the introduction of the infection and the onset of the disease, but gives us our first clew to the constancy of the period of incubation of certain maladies.

ANTIBODIES.

We showed in our first publication in 1906 that the mechanism of anaphylaxis did not depend on an antibody free in the blood serum and body juices, as is the case in diphtheria and tetanus. In other words, this phenomenon is not simply a neutralization of a soluble poison by a soluble antipoison. There are antibodies other than free receptors which are readily demonstrable *in vitro* and *in vivo*. The definition of an antibody still lacks precision, and the action of these substances is not clearly understood. In this state of our knowledge it would be dogmatic to insist that the phenomenon of hypersusceptibility depends on the action of an antibody. The indications, however, seem to be that we are dealing with such an agent.

The anaphylactin readily demonstrable in the blood of a guinea-pig many months after the introduction of a minute fraction of a cubic centimetre of horse serum subcutaneously, can hardly be a portion or "rest" of the original minute amount

of foreign protein injected. It seems probable that the introduction of the foreign protein stimulates or calls forth the production of anaphylactin, which flows free in the blood serum in relatively considerable amounts. There is evidence to indicate that anaphylactin is present in the blood serum in amounts greater than the amount of foreign protein originally introduced into the body.

We have shown that anaphylactin is present in the blood serum of immune guinea-pigs. This observation is very significant in this connection, for it indicates that the cells are protected despite the fact that they are continually bathed with the serum containing the sensitizing principle. The readiest explanation of this form of cellular immunity is the conception of a neutralizing antibody.

The further fact that hypersusceptibility, and not immunity to anaphylaxis, is transmitted from mother to young is another indication that we are dealing with an antibody.

Vaughan, Lewis, Nicolle, Besredka and most others—except Gay and Southard—who have studied this phase of the question consider an antibody necessary to explain the intimate nature of the phenomenon.

RELATION OF ANAPHYLAXIS TO PROTEIN METABOLISM.

It is interesting to note that Ehrlich's explanation of antitoxic immunity was based on a chemical conception of protein metabolism. Ehrlich's haptophore side-chains that seize the toxin molecule have the same or similar affinities as those that seize and combine with the protein food molecule.

Another great conception of immunity, developed by Metchnikoff, is also clearly associated with protein metabolism or cellular digestion.

We now have another conception of immunity in certain infections based on the action caused by the introduction of a foreign protein into the body. This view is also closely bound up with the subject of protein metabolism. It cannot but excite our wonder that the chemistry of the body should be so delicately balanced that the introduction of 1/10,000,000 part of a

gramme of foreign protein should be able so profoundly to influence it as to result in serious symptoms when injected a second time.

The whole problem of protein metabolism seems to be an adjustment in the sense of a defence. The power to assimilate and use foreign proteins is not achieved without a certain amount of violence to the body. The relation between the fundamental facts of protein metabolism and the immunity to certain diseases becomes clearer in the light of observations upon anaphylaxis. Work on both these problems will throw light on the fundamental processes of each other.

RELATION OF ANAPHYLAXIS TO ENDOTOXINS.

The fact that the great majority of bacteria do not produce soluble poisons such as diphtheria and tetanus has led to the belief that in such cases we are dealing with an "endotoxin." The endotoxin has long been regarded as a poisonous substance so intimately associated with the cell that it is not released until the microbic cell is broken up in the body. The inability to demonstrate these endotoxins has cast a doubt on their existence and increased the mystery of their action. It now seems probable that the studies on anaphylaxis may throw light upon this question.

When bacteria grow in the body they are dissolved by lytic agencies and the foreign protein in the individual germ cells may sensitize the body and afterwards poison it. The bacterial proteins may not be poisonous in themselves in the sense of an "endotoxin." We have, in fact, shown that protein extracts of bacterial cells at the first injection may produce characteristic symptoms, and this reaction may be followed by an immunity to the corresponding infection.

THE RELATION OF ANAPHYLAXIS TO TUBERCULOSIS.

The tuberculin reaction is one of the best known instances of anaphylaxis. Following a local infection with the tubercle bacillus the tissues generally become hypersusceptible to tuber-

culin. We have shown that a local hypersusceptibility may be produced by the direct application of tuberculin to certain tissues (conjunctiva). The same has been demonstrated for the skin, and is probably true of other tissues. This hypersusceptibility of the tissues immediately surrounding a tuberculous focus helps to encapsulate and limit the process. Should a tubercle bacillus lodge in or on a tissue in a state of tuberculin anaphylaxis the result is that all of Nature's protecting agencies are quickly concentrated on the point where they are most needed. We conceive that this active power of reacting quickly is not only an important factor in individual prophylaxis against tuberculosis, but it is one of the important agencies which prevent the spread of the disease after it has obtained a lodgement in the body.

The normal individual does not react to tuberculin. The tuberculous individual reacts promptly, except in the final stage of the disease. The difference between the normal individual and the individual in the final stage of tuberculosis is that the former has not had his anaphylactic powers developed, while the latter has had them developed and exhausted. A tuberculous individual in whom the specific power of hypersusceptibility to the poisons of the tubercle bacillus is broken down presents little or no resistance against the advance of the infection.

We may adduce a practical lesson from this: When tuberculin is used in large or too oft-repeated doses there is a tendency to break down or to exhaust the useful and beneficial hypersusceptible state of the tissues. In accordance with this line of reasoning, therefore, tuberculin would be of benefit in tuberculosis only when used in such a way as to develop and not diminish the power of anaphylaxis of the tissues. This explanation has been borne out in the use of tuberculin.

While all of the views expressed here are based on experimental data, our interpretations of them may not all stand the test of time. It is evident, however, that studies on the subject of anaphylaxis give us a broader and deeper insight into some of the difficult and abstruse problems connected with

both the recurrence of and the resistance to disease. The subject has proved a fruitful field for research. It has more than an academic interest. There is hope that a final solution of the mechanism of anaphylaxis will have a practical application in the treatment and prevention of a number of diseased states.

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THE SIGNIFICANCE OF OSMOTIC MEMBRANES IN HEREDITY*

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THE problem as to the nature of the processes that constitute osmosis is one that is of paramount importance in physiology and biochemistry and it is, in consequence, to-day receiving a degree of attention which is a measure of the appreciation which is accorded to it as a factor in explaining some of the fundamental phenomena of life. The problem is, however, not confined in its interest to physiologists and biochemists, for the physicist is attacking it, and the physical chemist has, in the last twenty years, with his theories of the gaseous condition of substances in solution and of the electrolytic nature of solutions of inorganic compounds, given a powerful stimulus to investigation of the problem. Indeed, because of the brilliancy of the speculations of physical chemistry and of their application to physiological and biochemical phenomena, the explanation of osmosis offered by the physical chemist has attained a vogue that dominates all our ideas about its phenomena.

That, however, we are still far from a generally accepted explanation is only too evident from a critical examination of the literature which has lately appeared on the subject. Callendar[1] in an article recently published refers to the various explanations now current as (1) the gas pressure theory, (2) the surface tension theory, (3) the association or hydrate theory, and (4) the vapor tension theory; and he goes on to point out that probably all these theories possess some elements of truth and that they may present to some extent different

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aspects of the same phenomena. Though this enumeration does not include all the theories that are current as to the nature of the processes of osmosis it suffices to indicate how inchoate are our views on the processes and laws involved.

From the physiological side there is a tendency to adopt the gas pressure theory as accounting for all the phenomena of osmosis simply because it furnishes so acceptable an explanation of many facts in physical chemistry, although this theory fails utterly to account for many physiological phenomena, as, *e.g.*, the marked differences which may obtain between the composition of the renal secretion and the blood plasma from which the kidney elaborates it.

It is evident that the physiologist has not heard the last word from either the physicist or the physical chemist on osmosis, and, as he cannot wait till finality is attained on the subject in the other departments of science, it is incumbent on him to survey the facts as he knows them and, if it is at all possible, to formulate a view explaining and homologizing them in order that he may not find himself in an intellectual *impasse*. To face these facts and to draw conclusions from them that will at least for the hour prove acceptable is better than to leave the task to the physical chemist or the physicist who investigates but one aspect of the question. It is further to be noted that while the physicist and the physical chemist concern themselves with a very limited part of the question the whole range of physiology comprehends facts which are of a wider and more significant value than those the physicist and physical chemist find in the subject. In other words osmosis is a fundamental factor in the processes whose results are the vital phenomena we know, and in the play of this force, there is an infinite series of variations which never appear in the membranes which the physicist and physical chemist employ in their investigation. It is perhaps almost wholly due to the very narrow range of the problem as they attack it that the results which they obtain fail to be of service in explaining physiological osmosis. It is indeed not surprising that it should be so. The physicist and the physical chemist have not, as a rule, an

extensive acquaintance with physiological facts, particularly those of animal physiology, and they cannot understand why their explanations of the phenomena may fall short of acceptance by the physiologist. It is, of course, to be admitted that such explanations are received in vegetable physiology in which the cult of semi-permeability has prevailed for nearly half a century and in which the phenomena of osmosis are, superficially observed, of no wider range than they are in physics and physical chemistry.

It is necessary to get back to fundamentals in order to free ourselves from the confusion which has overtaken our ideas on osmosis. We must, if we would clearly understand what osmosis involves, put aside, for the hour, all the views current about permeable and semi-permeable membranes which have been advanced during the last forty years and we must endeavor to collate carefully all the facts bearing on the subject in order to reach a reasonably certain or safe conclusion. This makes it necessary for us to go back to the observations and conclusions of the earlier investigators of osmosis.

The phenomena of osmosis have not been known long. The first observation, that credited to Nollet, is of as late a date as 1748. The first observation that attracted any attention, however, was that of Parrot,[2] published some forty years later, which was made with a glass cylinder completely filled with alcohol, the mouth of which was closed with a bit of bladder membrane and the whole inverted in a dish of water. The water passed through the membrane, thus increasing the volume of fluid in the cylinder and consequently distending the membrane, from which on its being pricked with a needle a thin column of water and alcohol spurted several feet high.

Dutrochet and Magendie were the next to study the phenomena of osmosis and their first observations were published in 1826. Dutrochet [3] who introduced the expressions endosmosis and exosmosis, continued during the eight following years his investigations of the subject and in all his experiments employed the apparatus which we still know as the endosmometer. With this he determined when two fluids of different composition are

separated by a membrane in which direction the current proceeds through the septum and for this purpose he varied the composition of either fluid not only quantitatively but also qualitatively. As a result he established not a few of the facts of osmosis now known, such, for example, as that the current is a double one, that is, that there are two currents in opposite directions and that the temperature of the fluid influences appreciably this result, but he specially noted also, and this particularly in his last publication on the subject, that the composition of the membrane has a very important effect in determining not only what may pass through the membrane but also the direction of the predominant current.

These latter observations of Dutrochet have been overlooked in all the subsequent references to the literature of the subject, and as they are of fundamental significance it may be of service to recall a few of them.

Dutrochet found that when an animal membrane is used to separate an acid solution from pure water the current is from the former to the latter, but when a vegetable membrane is employed, as, for example, the shell of the bladder-nut (*Colutea arborescens*) or a large membranous leaf of the bulb of the leek (*Allium porrum*), the direction of the current was reversed, that is, it was from the water to the acid. Again, with animal and vegetable membranes separating water and alcohol the water passed through to the alcohol, but with a membrane made from light silk (taffeta) faced or impregnated with rubber the predominant current was in the opposite direction. Water did pass through the membrane, but only in small quantities. In this connection he pointed out that rubber is not permeable to water though it is permeable to alcohol, and this, he held, explains the reversal of the current and the penetration of water in the presence of alcohol, for the latter, penetrating the rubber membrane, attracts the water to the molecular interstices which are not accessible to the water alone, and this brings about the passage through the membrane of minute quantities of water.

That these appeared to Dutrochet as cardinal points in osmosis may be gathered from the fact that his last paper closes

with a statement emphasizing the importance of the composition of the membrane as the sole factor in determining osmosis.

Dutrochet noted also the curious point that when oxalic acid or tartaric acid in solution is contained in the endosmometer the diffusion current instead of passing through the membrane to the acid goes in the opposite direction and in consequence the pressure becomes negative.

Brücke [4] followed Dutrochet in 1841 and confirmed many of the latter's observations and particularly those based on the differences in the composition of the membrane as modifying or reversing the direction of the predominant current. Later [5] he corroborated Dutrochet's observations on the occurrence of a double current in osmosis, repeated his experiments with alcohol and water separated by bladder membrane and by rubber septa, and accepted Dutrochet's explanation of the results, although he has been credited by subsequent writers on the subject of osmosis as postulating a system of pores in the membrane through which the diffusion obtains.

It is curious to note that Brücke, a quarter of a century before Traube, was the first to perform an osmotic experiment with a precipitation membrane. One experiment made by him is worthy of description here. He closed one end of a glass cylinder with a piece of bladder, lowered the closed end into a concentrated solution of lead acetate, and after the membrane became saturated with the lead salt poured into the cylinder a saturated solution of potassium bichromate diluted with one-third of its volume of water. There developed at once a precipitate of chromate of lead in the membrane, but both fluids remained quite clear. To demonstrate that the membrane was still permeable, sugar was added to the lead solution with the result that for several days afterwards its volume increased at the expense of the volume of the bichromate solution. There passed from the latter only pure water or water containing traces only of potassium acetate. If the position of the fluids as regards the membrane were reversed, the lead salt solution now being inside the cylinder, the addition of sugar to the bichromate solution caused a movement of water from the lead

salt solution to the bichromate solution. All the results showed that the membrane was permeable to water in either direction, yet not to the salts, but Brücke did not determine whether any of the sugar was transferred through it.

Kürschner [6] summarized the results of an extensive series of experiments, made not only with liquids but also with gases, to the effect that it is the moisture in the membrane which determines the diffusion through it of solutes or solvents on either side of it and that the substances, whether solute or solvent, or both, diffuse through the membrane because they mix or combine with the fluid moistening the membrane. The strength of the currents varies according as the affinity of the fluids for the substance of the membrane varies.

Liebig [7] attributed osmosis to diffusion. Membranes absorb different quantities of various fluids. If then a membrane separates two fluids it will unite with them and in consequence, in the case of alcohol and water, the alcohol which bathes one of its faces will remove by diffusion some water with which it will not be saturated, so that it will absorb a further quantity which will be removed in its turn and thus will be established a current from water to alcohol. A like effect is produced by the alcohol on the water, but as the membrane absorbs the alcohol more actively than the water the current of water to the alcohol will be more rapid than that from the alcohol to the water.

Graham,[8] who was the first to introduce the expression osmosis, as the result of his observations came to the conclusion that a change in the membrane is a condition indispensable to the occurrence of osmosis. According to his view one of the faces of the membrane is acid, the other basic. This results in a continual change in the composition of the membrane. When the septum separates pure water from a solution of an acid or of a base the water plays the rôle of base in one case and of acid in the other. In every case the flow of fluid is towards the basic side of the membrane and this suggested to Graham an analogy with electrical endosmosis in which water follows hydrogen and alkali in the direction of the electrical current.

With this explanation he met the difficulty presented by the results obtained by Dutrochet with oxalic acid and tartaric acid. These when in solution give a negative pressure, that is, they diffuse more readily through animal membranes into pure water than the latter does in the opposite direction even though it is under greater pressure. This phenomenon Graham named negative osmosis. He found it obtained with solutions of citric acid and hydrochloric acid, as well as with the two acids already mentioned, and it obtained also with the chlorides of gold, tin, and platinum and with nitrate of magnesium.

It is rather curious to note here in passing that in his elaboration of his theory of osmosis Graham [9] foreshadowed the hydrate theory of solutions of to-day and also anticipated in a measure the hydrone form of it advanced by Armstrong.

Lhermite [10] substituted for membranous septa between two fluids a liquid in which both fluids are soluble but in different degrees, the two fluids also being soluble in each other. Thus, when in a cylinder water is first added, then turpentine, and finally alcohol, the layer of turpentine separating the two other fluids is capable of dissolving in itself quantities of either and thus an exchange between the water and alcohol takes place. As the alcohol is much more soluble in turpentine than is the water the alcohol will eventually be found in the water, and the layer of turpentine will be on the surface. If castor oil is the separating liquid the same result is obtained.

The part played by membranous septa, according to Lhermite, is none other than that illustrated by the liquid septa. The membrane has an affinity for one or both of the fluids or their solutes, that is, the fluids, or their solutes, or both, are soluble in the membrane, but in different degrees, and in consequence a preponderating current is set up in one direction. Lhermite described a striking experiment confirming the view that osmosis is based chiefly on solubility in the membrane. As is well known, when a porous porcelain septum separates alcohol and water the osmotic current is from the water to the alcohol. Lhermite impregnated the wall of a porous porcelain vessel with castor oil, poured water therein, and placed the vessel in

alcohol. The current then passed from the alcohol to the water. He explained the result by pointing out that water is not, but alcohol is, soluble in castor oil and thus the alcohol can get through to mingle with the water but the latter cannot transverse the oil. In consequence the direction of the current was reversed.

Poisson [11] shortly after the publication of the earliest of the results of Dutrochet's studies advanced the view, based on purely theoretical grounds, that the phenomena of osmosis can be explained as due to capillarity. Operating in and through the pores and capillary passages of the membrane, the capillary attraction differs on the two faces of the membrane owing to differences in composition of the two fluids and, in consequence, the greater current would pass from that face where the capillarity is greater to that where it is less. This explanation, accepted by Magnus [12] and Fischer,[12a] was rejected by Dutrochet and Graham, but Lhermite accepted it as a force in osmosis supplementing the dissolving power of the membrane.

The discussion of the nature of processes involved in osmosis assumed quite a different character after the publication of the results of the observations of M. Traube and Pfeffer on precipitation membranes, and as these results have proved to be of importance far beyond the significance attributed to them at the time by these investigators the dates of their publication, 1867 and 1877, have marked out so striking an epoch in the history of the subject that it has practically led to an ignoring of the work of earlier authors. This has had the unfortunate result that some of the previously ascertained facts, *e.g.*, of Dutrochet, Brücke, Kürschner, and Lhermite have been again put on record as if they had not been previously known. Had those facts been adequately known to some of the later investigators it is doubtful if a number of the current concepts of osmosis and particularly that of the membrane as a sieve-like structure would ever have been advanced.

One of the concepts which the work of the later investigators has rendered prominent is that of the semi-permeable membrane, and in all the speculations of the last thirty years on the

subject of osmosis it has occupied a central point, so that it has now become almost a commonplace in physiological text-books and treatises.

This concept postulates a diaphragm which is freely permeable to water but not to any substance dissolved in it. If, for instance, sugar is the solute, the membrane will not permit it to pass through to the other compartment but the water of the latter passes through to dilute the solvent of the sugar. The membrane in this instance is so constituted as to retard completely the passage of the molecules of the solute but not those of the solvent.

Traube attributed the permeability to the magnitude of the interstitial spaces between the molecular aggregates forming the precipitate membrane being such as to allow the molecules of the permeating substance to pass readily, but the diameter of these spaces he believed to be less than the diameter of a molecule of either of the solutes going to form the precipitate, while Pfeffer freely recognized that permeability involved imbibition of the membrane by the permeating substance (1) either passing into and through the molecular interstices or (2) dissolving in the molecules of the precipitate membrane, thus finding its way through the latter. His explanation has, however, been ignored in the development of the theory of the gaseous condition of solutes in relation to osmosis for it has been generally assumed that the membrane is a passive structure fashioned more or less like a sieve.* That this is a correct representation of the view current amongst not only the adherents of the gas pressure theory of osmosis but also others, including physicists, may be gathered from the fact that in the last ten years it has been specially remarked by various investigators (Barlow, Flusin, Nernst, and others) that solubility in the substance of the membrane itself is a factor in osmosis.

The concept of a semi-permeable septum first acquired importance from the results of Traube's observations[13] on mem-

* See on this Höber, Physikalische chemie der Zelle und der Gewebe, Zweite Aufl., p. 166.

branes formed of two substances in solution interacting so as to form a precipitate derivable from both. A typical example of such a membrane may be obtained in the manner described by him. A piece of glass tubing about six or more inches in length, open at one end but covered at the other by a piece of rubber tubing compressed with a clip, is taken and into this is drawn a few drops of a 2.8 per cent. solution of cupric acetate. The open end is now lowered into a test-tube containing a quantity of a 2.4 per cent. solution of potassic ferrocyanide. The liquid in the inner tube is, by shifting the pressure on the rubber tubing, made to form a plane surface at the mouth of the tube and cupric ferrocyanide is deposited there as a fine transparent film which closes the opening. This film or membrane prevents the diffusion of the copper salt downwards and of the potassium ferrocyanide upwards, as shown by the facts that the cupric ferrocyanide does not occur above or below the film and that the film remains for a considerable time transparent and of very great tenuity but yet of sufficient density and grain to prevent either salt passing through it.

That water, however, does pass through such a membrane can be demonstrated by showing that the refractive index of the solution in the immediate neighborhood of the film is increased, this indicating that the concentration of the solute is increased at that point, or it may be demonstrated by placing a crystal of the chloride or sulphate of copper in a moderately concentrated solution of potassium ferrocyanide. In this latter case there is formed a precipitate of cupric ferrocyanide in the form of a membrane about the crystal which distends gradually, obviously only by the penetration of water alone, for the distention may result in a rupture and then there escapes a drop of concentrated solution of chloride or sulphate of copper, which in contact with the external fluid gives a new precipitate repairing and thus closing the rupture in the original membrane. Through this also the water passes and distention continues till the osmotic pressure inside and outside is the same.

These films are, however, so delicate and so easily ruptured that they cannot be made to furnish data which would enable

us to determine the pressure exercised by the dissolved substances.

Pfeffer[14] met the difficulty which they present by causing the precipitate of cupric ferrocyanide to be deposited in the walls of unglazed porous earthenware or porcelain vessels. This he succeeded in doing by pouring one of the solutions, *e.g.*, the cupric sulphate, into the porous vessel, which was then placed in the other solution. The two solutions diffused through the pores of the wall of the vessel and met, forming a precipitate film which, owing to the firm support afforded by the wall, could not be displaced. After washing away and dissolving out all traces of the two solutions the porous pot was made to serve as a membrane to determine the pressure exercised by dissolved substances. This was done by putting into the porous receptacle some of the solution which required to be examined and closing the vessel with a firmly fitting stopper through which penetrated a manometer connection to indicate the pressure. When the vessel is placed in distilled water the latter passes through the wall of the porcelain tube and as there is apparently no corresponding movement in the opposite direction the pressure inside rises and is registered by the manometer.

With such membranes Pfeffer obtained some striking results, but the chief one was that the pressure was found to be dependent on the strength of the solution, being almost proportional to the concentration in the case of organic solutes like cane-sugar and dextrose and also in proportion to the rise of temperature, but in the case of salts the results were not so constant.

Pfeffer did not account for the causation of his results and it was van 't Hoff who was the first to offer an explanation of them. This explanation practically embodies the now well-known theory of the gas nature of solutions, which is accepted widely as a cardinal principle of physical chemistry.

Pfeffer in postulating the existence of a semi-permeable membrane was obviously led by a desire to explain how it happens that the living cell and, particularly, the vegetable cell,

placed in aqueous media maintains its normal turgor due to a high hydrostatic pressure and at the same time retains all its organic constituents, dextrose for example. The facts then known apparently indicated the existence of a membrane which allowed water and salts to enter the cell but not the organic contents to escape. Such a membrane implied a semi-permeable character.

There are, of course, physical phenomena which might in themselves have suggested a parallel explanation, and one of these is manifest in the action of palladium on hydrogen.[16] At ordinary temperatures palladium absorbs hydrogen but gives it up again when heated in vacuo to temperatures above 100° C. Palladium does not so act towards any other gas and in consequence at about 200° C. it can be constituted as a membrane permeable of course to hydrogen but unpermeable to carbon monoxide, carbon dioxide, or nitrogen.

If any of these gases be contained in a palladium tube surrounded with hydrogen the latter will pass through the palladium and cause an increase in pressure in the tube equal to 90 per cent. or more of that of the external hydrogen.

According to Richardson, Nicol, and Parnell[18] platinum also acts like palladium. The hydrogen, as may be gathered from the observations of Hoitsema,[17] Krakau,[18] and others, is absorbed not in the molecular but in the atomic condition; it enters as a solute in the metal and it does not form a compound with the latter. The palladium, from this, would appear to dissolve readily the small quantity of the ionized hydrogen and to liberate it as H_2 in the interior of the tube. As the amount of dissociated (ionized) hydrogen as compared with that in the molecular condition must at any moment be very minute it is obvious that palladium is extremely receptive as a solvent of ionized hydrogen, and this activity therefore constitutes a very important factor in the exchange of hydrogen between its two surfaces. Accordingly the concept of the palladium as a sieve must be set aside.

Even, however, when we are dealing with precipitate membranes we must discard the sieve explanation and attribute

selective activity to such septa. Barlow [19] has pointed out that the ferrocyanide membrane, under mechanical pressure, is more readily permeable to alcohol than it is to water, that is, the larger molecules pass through more readily than the smaller. He found also, as did Dutrochet, Brücke, and others before him, that when gutta percha membrane separated pure alcohol and pure water the alcohol passed through to the water, the reverse of what happens when a copper ferrocyanide membrane is used. Pringsheim [20] by varying this method of forming the precipitated membrane, obtained some interesting results. He took a U-shaped tube into which he poured enough liquefied gelatin to fill the bottom and lower portion of each limb of the tube. On this "setting" he poured into one limb, a solution of potassic ferrocyanide and into the other a solution of sulphate of copper. The two solutions diffused into the gelatin and towards each other, forming where they met a regular continuous well-supported membrane. From his experiments with this membrane he concluded that its permeability does not depend on its chemical nature but on the concentration of the salts contributing to its formation. If the cupric sulphate is in excess the membrane formed is permeable to it alone, but if the potassium ferrocyanide is in excess the membrane is permeable only to this salt. It is, however, not the size of the molecules which determines whether they shall go through the membrane, it is rather more or less the affinity of the dissolved substances for the material constituting the membrane that is the deciding factor.

It must not be overlooked that the term semi-permeable has been applied to membranes other than the precipitated ones. An instance is that of Nernst.[21] He used pig's bladder to separate a saturated solution of water in ether from a quantity of the same solution in which, however, benzine was dissolved. The membrane allows the ether-water to pass through it but keeps back the benzine. Another example of a so-called semi-permeable septum differing from the precipitates is that of Raoult,[22] who employed a rubber membrane to separate ether and a solution of ether in methyl-alcohol. In this experiment

the ether but not the alcohol passed through the membrane. A like septum was employed by Flusin who found that it was "permeable" to carbon bisulphide, chloroform, toluol, ether, and every organic fluid which dissolves caoutchouc. The velocity, however, of the diffusion through the membrane varied almost directly according to the solubility of the diffusing compound in the rubber. All these results distinctly point to an action on the part of the membrane which is not ordinarily postulated in the semi-permeable membrane of the physical chemist. The rubber dissolves the "permeating" substance and, in the case of the pig's bladder used by Nernst, the substance of the bladder membrane dissolves the water-ether solution but does not dissolve the benzine, and hence the latter is held back.

It is obvious that if this view is correct the currently received explanation of osmosis is defective.* This postulates that the solute is in a state of gas, the molecules and the dissociated ions of which bombard the membrane and the free surface of the solution, and this bombardment tends to extend the limits of the solution, which can only happen if the membrane permits the molecules or ions of the solvent in the other compartment to pass through it. This theory leaves out of

* The difficulty in the way of accepting wholly the gas theory of solutions has not been lessened by some of the results of the investigations published during the last three years. Morse and Fraser (*Am. Chem. Journal*, 1905, xxxiv, 1), working with precipitate membranes, found that in the case of concentrated solutions of cane sugar (*e.g.*, 342.22 Gm. in 1000 Gm. of water, that is, 283.45 Gm. in 1000 *c.c.* of solution) the pressure found is out of proportion to the concentration, that, for instance, the 283.45 Gm. in a litre of solution exercised a pressure which according to the theory should be exercized only by 342.22 Gm. in 1000 *c.c.* of the solution at the same temperature. Lord Berkeley and Hartly (*Phil. Trans.*, 1906, ccvi, 481), also using precipitate membranes, found in the case of cane-sugar, dextrose, and galactose that while dilute solutions gave pressures corresponding to the Boyle-Avogadro law, concentrated solutions gave pressures widely departing from it. Further, Kahlenberg (*Journ. of Physical Chem.*, 1906, xx, 141), using rubber membranes with $\frac{N}{4}$ solution of cane-sugar and of lithium chloride in pyridine, found that the latter gave a little more than half the pressure obtained with the cane-sugar, while according to theory both solutions should give the same pressure.

account activity on the part of the substance of the septum * and yet such activity may be the only factor in such osmosis, as it certainly is in the case of hydrogen diffusing through palladium into a medium of nitrogen. Here the bombardment would be absolutely ineffective if the palladium did not dissolve the ionized hydrogen. Even in ordinary aqueous solutions this bombardment must be ineffective, for it has been estimated by Nernst that to drive one gramme of urea through water at the rate of 1 cm. a second a pressure is required equivalent to 40,000 tons weight, and yet in a gramme-molecular solution of urea the pressure according to the gas theory of solution is 22.4 atmospheres or about 336 pounds to the square inch.

The difficulty of accounting for some of the phenomena of osmosis on the current conception of a semi-permeable membrane and the gaseous condition of the solute has led a number of physicists and physiologists to reject both as quite inadequate and unsatisfactory. Isidor Traube [23] advanced the view that all the phenomena of osmosis are due to difference in surface tension on opposite sides of the septum, the difference determining the direction and velocity of the osmotic current, the direction being towards the liquid having the greater surface tension. He claims, however, that "the difference between the surface tensions can not be considered as equal to the osmotic pressure for this new pressure is quite different for isomotic solutions."

More recently in a long paper [24] containing the results of experiments bearing on the point he has restated his view "that the osmotic and capillary relations of solution, without exception, run parallel," and further that substances, like inorganic salts, which increase surface tension do not permeate cells, while those which lower surface tension do, and the more so the greater the molecular depression of surface tension they produce. In consequence the number of particles ("Teilchen") of the solute is not the only factor in osmosis as postulated in

* Max Roloff, for instance, in Koranyi and Richter's *"Physikalische Chemie und Medizin"* (pp. 143–4) asserts that the membrane has upon the distribution of the solutes or the solvents "keinen Einfluss."

the theory of van't Hoff, since the energy ("Haftdruck") in the form of surface tension which the dissolved molecules and ions develop in the solution is an important element in the phenomena. It is only when this energy is equal on both sides of the membrane that one can, in the case where the two solutions contain like numbers of particles, speak of equilibrium in osmotic pressure.

That surface tension is the cause of osmosis is also the view of Batelli and Stefanini [25] who, however, hold that the current does not necessarily move toward the fluid having the higher surface tension but always in such a direction as to make the surface tensions on both sides of the septum the same.[26] Barlow found in some of his experiments that the current passed from the weaker solution, that is from the one having the greater surface tension, to the more concentrated solution and, therefore, he does not support Traube's view. He suggests that the necessary factor in osmosis is "that the membrane must absorb that liquid which in going through, forms the osmotic current."

Flusin [27] also criticizes the conclusions of Batelli and Stefanini and denies as contrary to facts, that the direction and force of the current in osmosis depends only on the variation in the surface tensions of the liquids and not at all on the nature of the membrane. In support of this he refers to the different results when alcohol and water are separated in one case by pig's bladder, in another by a rubber septum. He points out also that when equimolecular solutions of salicine and cane-sugar are separated by a precipitate membrane of cupric ferrocyanide of a character unpermeable to both solutes they remain in equilibrium although, according to the Italian observers, salicine lessens and cane-sugar increases surface tension. Further in the case of negative osmosis first observed by Dutrochet and studied by Graham, as exemplified in aqueous solutions of tartaric acid separated from pure water by pig's bladder, the acid solution which has the lower surface tension flows through the membrane to the fluid which has the higher surface tension.

That surface tension does play a very considerable part in osmosis, may be found on consideration of some elementary facts in connection with solution. In every vessel holding a fluid there are two tensions, one at the free surface, the other where the liquid is in contact with the wall of the vessel. Whenever the former increases the latter diminishes. It has been observed that for most salt solutions the tension at the free surface is greater than in the case of distilled water. Hence the other tension in such salt solutions, that between the liquid and the wall, must be decreased, and in consequence the layer of liquid in contact with the wall will become richer in salt than the rest of the solution.[28] When the solution moves through capillary tubes along a filter or upwards through finely divided quartz the salt will collect on the capillary walls, on the fibres of the filter, or on the particles of quartz, and the current, as it ascends, will become more and more dilute until finally it is distilled water. J. J. Thomson and Monckman filtered potassium permanganate from its solution by passing it through finely divided silica and a similar separation may be obtained by allowing dilute permanganate solutions to diffuse into filter paper. Perhaps the most striking illustration of this phenomenon may be obtained by suspending long strips of filter paper so as merely to dip into a solution of copper acetate. In a few minutes the fluid runs up several inches but the upper half-inch or inch of the moistened portion does not contain a trace of the copper solution, as may be shown by treatment with potassium ferrocyanide solution or ammonium sulphide.

When, in consequence, a septum is in contact with a solution whose surface tension at the air contact is high, the solute will tend to condense on the surface of the septum while the solvent will diffuse through from the other compartment to dilute the concentrated fluid. This would cause the retention of such substances as sugar and urea, which lower surface tension, at the air contact, while permitting their solvent to diffuse.

It must be admitted that surface tension is not a factor sufficient to explain all the phenomena of osmosis, and especially those manifested in cellular absorption and diffusion. It will

not explain the absorption of colloids in the intestine, the passage of fats and proteins through the endothelial lining of capillaries and the diffusion through living membranes of material which is not in solution but rather in suspension as colloids. The difficulty of explaining these and the inadequacy in this respect of the concepts derived from the semi-permeable membrane have promoted in recent years revival of the old doctrine of vitalism as a distinct force concerned in the exchange of material through the septa formed by living cells. To accept an hypothesis postulating the existence of a biotic or vital force distinct from the physical or chemical forces that we know and can examine, is, I think, to despair of an intelligible solution of the problem, and it does not seem justifiable as yet to adopt that attitude, the more so since the range of facts bearing on the problem has been recently greatly widened.

Overton,[23] who has investigated at great length the velocity with which chemical compounds diffuse into living cells, has formulated conclusions which associate this diffusion with the so-called distribution coefficient. By this term is meant the constant relation in which a substance independent of its quantity at a definite temperature distributes itself between two different solvents which are in contact. An example of such a substance is succinic acid, which has a distribution coefficient between ether and water of 5.2, that is 5.2 times as much of the acid dissolves in the ether as is taken by the water.

Overton found that in general various compounds pass through membranes the more soluble they are in such substances as fats, cholesterin, lecithin, etc., and he holds in consequence that these lipoid bodies in cells are the cause of the diffusion into the latter of various lipoid-soluble substances, and further, that the magnitude of the distribution coefficient between fat, lecithin, and cholesterin on the one hand and water on the other determines the velocity of osmosis. The lipoid material in a membrane takes up a solute from a fluid bathing it at a velocity proportionate to the distribution coefficient and at the

same time the substance is passed on from the membrane to the interior of the cell.

In illustration of Overton's theory it would follow that a substance soluble in water but not, or scarcely, in oil would not pass through cell membranes readily. For example, glycerin presents this feature and it penetrates cell-protoplasm slowly. The monochlor compound of it, monochlorhydrin, is soluble in fat and diffuses into the cell quickly, but the dichlor compound, dichlorhydrin, which is extremely soluble in fat, almost instantaneously penetrates cellular elements. One may parallel these results with those obtained with the use of urea, monomethyl urea, dimethyl urea, and trimethyl urea, all of which have solubilities in fat increasing in the order named and with corresponding power to penetrate cell-protoplasm. On the other hand, many very active electrolytes are insoluble in fats and it is found that they penetrate slowly the cell-protoplasm.

That liquid material does obtain in cell-membranes seems to be indicated by the results of *intra vitam* staining. Overton found that the dyes which stain the living cell are those, as a rule, which are soluble in fats, cholesterin, and lecithin, in which the dyes incapable of producing a vital stain do not dissolve.

One of the most noteworthy points in Overton's generalizations is the remarkable fact that all the principal narcotics, anæsthetics, and antipyretics are rapidly diffusing substances. This fact was also pointed out independently by Hans Meyer, and both he and Overton hold that the efficacy of a narcotic depends on its solubility in lipoids.

These speculations of Overton, while very interesting, do not solve the problem of osmosis wholly, for they offer in themselves difficulties which Traube has pointed out.[30] For instance, if there must be solution in the lipoid material of the cell-wall before the substance can enter the cell-protoplasm, then even the most rapid osmosis would be a very slow process. Further, it is impossible to understand why this lipoid material should not hold this dissolved material all the more tenaciously the

more forcibly it has attracted it, instead of passing it on to the interior of the cell. It is above all impossible, Traube holds, to understand how water can penetrate lipoid-holding membranes since it is not soluble in lipoids; and further, salts, as in the case of renal and other secretions, readily penetrate cells, which is quite irreconcilable with Overton's theory.

Moore [31] has also criticized the lipoid theory of osmosis in practically the same terms as Traube, pointing out also that it does not furnish any basis of explanation of how energy is expended in concentrating any secreted or absorbed substance, and he also holds with Traube that a lipoid as a good solvent for a given constituent does not give it the power to pass that substance through the cell in a more concentrated condition, or indeed to alter the concentration of the solute anywhere save in the solvent itself.

The criticism of Traube that a lipoid-holding membrane is impermeable to water will not hold wholly, for lecithin, cerebrin, and protogon swell up greatly in water and they are consequently permeable to water, but Nathansohn [32] points out that when lecithin swells up in water it thereby loses the capacity to dissolve the substances soluble in lipoids. He, however, would still accept Overton's theory but would modify it to account for the diffusion through lipoid-holding membranes of both water and lipoid-soluble material. This he does by postulating that the membrane is a composite or mosaic structure in which a portion of the component elements consist of unswellable cholesterin impermeable to water and the remainder of protoplasmic material which has the properties of a typical semi-permeable membrane.

In support of this view of Nathansohn there are facts which Pascucci [33] has determined. The latter found that the stromata of red blood-cells is constituted, one-third of lecithin and cholesterin with a minute quantity of a cerebrin-like compound and the remainder, two-thirds, of proteid. As the stromata consist in large part of the membranes the postulated mosaic structure of Nathansohn may occur in red blood-cells at least.

The very fact that we are driven to postulate a mosaic mem-

brane in order to apply Overton's theory indicates how inadequate the latter is in explanation of osmotic phenomena. That lipoid substances can facilitate the exchange between the exterior and the interior of a cell may be admitted, but that it is the only factor or the most important factor is less acceptable, and one must trust to a larger conception of osmosis in order to be in a position to understand its phenomena.

This larger conception may be gleaned from the results of the studies of Kahlenberg.[34] In order to understand rightly the significance of his observations one must, to a certain extent, disregard the distinction between colloids and crystalloids which has been held valid since Graham's time. We must recognize that a substance which acts as a colloid to water is not necessarily a colloid to every other solvent. Indeed there is evidence that in the case of some of the typical organic colloidal solutions in addition to the ultra-microscopic suspension particles present there is a quantity of the same material in the solution side by side with the particles. Hardy has shown this in the case of agar and gelatin; and the phenomena associated with all colloids except those of the inorganic class seem to indicate that there are two phases in all such suspensions, namely, the solid-water phase, which enters into the constitution of the suspension particles and the water-solid phase, which forms a true solution. When the latter is eliminated there is brought about a conversion of some of the solid-water phase into the water-solid phase until equilibrium is established. It is, therefore, not correct to speak of colloids as wholly suspensions.

On the other hand the very fact that a membrane does not allow the passage of a so-called colloid through it is no evidence that the latter is wholly a suspension, for if the composition of the membrane is rubber it will keep back even sodium chloride, that is, a crystalloid. This shows how artificial is the distinction which we obtain by using one kind of membrane as a means of distinction between suspension and solution. If we wish to understand the phenomena of osmosis we must discard all the concepts which we have gained from the results obtained with the ordinary dialyzing membrane formed of parchment. It

would be just as reasonable to classify all substance into two groups in their relation to a rubber membrane, for neither rubber nor parchment membranes exist in nature, and those membranes which do exist are not of the kind that make a distinction between the so-called colloid substances which are formed by, or are the products of, tissue and cellular activity; there are none that are insoluble in the fluids bathing such tissues or cells or in the intracellular fluids. To express it in another way, the reason why substances which are concerned in the vital process circulate and diffuse in tissue elements is that the physiological fluids, extracellular as well as intracellular, are so composed as to dissolve them.

To return to Kahlenberg's observations, it may be pointed out that while he was not the first to use rubber membrane as septa for osmotic experiments, since Flusin, Raoult, Tammann, and others had done this before him, he was amongst the first to investigate thoroughly the properties of such rubber septa and to indicate the significance of the results.

One may not detail fully the results of his observations, and those who are interested in the study of osmosis may, with profit, consult the original paper, for it is replete with descriptions of experiments which illustrate osmotic phenomena which cannot be obtained with parchment or precipitate septa and which help to clear up some of the chief difficulties experienced in the past in attempts at explaining physiological osmosis. I shall here give only a few details to illustrate the significance of Kahlenberg's results.

In the first place he found that the composition of the membrane is a decisive factor in osmosis. He used in his experiments, amongst other membranes, septa of pure rubber and various fluids as solvents, but the most striking results were obtained with pyridine. With pyridine only on one side of the septum, but with the same medium containing cane-sugar and copper oleate on the other, it was found that the colloid, copper oleate, freely passed through the septum, but the crystalloid, cane-sugar, remained behind. On the other hand when two crystalloids, camphor and cane-sugar, were in solution

in pyridine, the camphor, but not the cane-sugar, passed through the membrane. Here two crystalloids were separated from each other by osmosis. Further, Kahlenberg, as he stated in an address delivered to the Chemical Section of the American Association during its last session, has been enabled by this method to separate two colloids from each other.

The rubber membranes in Kahlenberg's experiments were not wholly impervious to sugar, for traces of it passed through them and so also did nitrate of silver and lithium chloride, but in very much smaller proportions than in the case of cane-sugar. That means that the non-electrolyte sugar diffuses through the membrane more readily than either of the electrolytes.

When instead of rubber septa, which he found permeable to copper oleate dissolved in benzine, he used a parchment septum with benzine on one side and benzine containing copper oleate in solution on the other no penetration of the membrane occurred; that is, the colloid copper oleate which passed through the rubber septum does not pass through parchment.

These and other results of Kahlenberg's observations make it quite clear that the membrane is not a passive element in osmosis. In the case of rubber septa with pyridine only those substances would pass through which are soluble in hydrocarbons whether they are crystalloids or colloids. "The current view that crystalloids always pass through membranes more readily than colloids," he says, "is evidently untenable, as it has been shown that just the opposite may occur and that even crystalloids may be separated from each other by dialysis when the proper septum is chosen. *Whether substances can be separated by dialysis or not does not depend at all on their crystalline or non-crystalline nature, as is so commonly supposed, but upon their affinity for the septum employed.*"

Of course, there are in nature no membranes like in composition to rubber, any more than there are membranes like parchment, or like the precipitated membranes of Traube and Pfeffer, but the one distinctive point obtained from the use of rubber septa which membranes of other composition do not

permit us to ascertain *is that osmosis is due to the solvent activity of the membrane whatever its nature. From this we may conclude that the composition of the membrane and the solubility in that membrane of the solutes on either side of it are all important factors in determining whether diffusion shall obtain.*

With these generalizations and with a new viewpoint we are in a position to appreciate and understand some problems which have had their origin in the results of some investigations of my own on the microchemistry of the cell and which I believe have a profound significance in relation to heredity.

It is well known that caseinogen and egg albumen when put in a loop of the intestine are absorbed apparently unchanged and excreted by the kidneys. When the same proteids are intravenously injected they are then also excreted by the kidneys. In the former case the mucous membrane of the intestine itself, composed of "colloidal" material, takes up the "colloidal" proteids and passes them on into the blood. In both cases the reflected epithelium of Bowman's capsule, itself also of "colloidal" composition, allows the foreign proteids to pass through with the water and salts. Again we know that the normal proteids and fats of the blood-plasma pass through the endothelial cells of the blood-capillaries to provide constituents of the lymph. The fats in the lymph are destined in part to penetrate the cytoplasm of certain connective-tissue elements to be deposited therein and from these intracellular deposits to diffuse away again according to the needs of metabolism. In this passage from blood-vessels into cells "colloids" diffuse through "colloidal" membranes and, as in the case of the caseinogen and albumins, the only explanation possible is that the membranes are of such a composition that they dissolve the water-solid phase of the "colloids" (proteids and fats) just as the rubber membrane in Kahlenberg's experiments dissolved the copper oleate or the camphor and passed it through to the other side.

It is, however, in cells themselves that we see striking evidence of diffusion of colloid through colloid. In the villi dur-

ing the absorption of fat the epithelial cells convey it to the underlying adenoid tissue and the latter pass it on to the lumen of the lacteal vessel. This transmission is not through the spaces or lacunæ of the adenoid tissue but through the colloidal substance of the adenoid trabecular network stretching between the basement membrane and the wall of the lacteal vessel, and it is transmitted dissolved in this "colloidal" substance. Indeed it penetrates the wall of the lacteal vessel only by dissolving in that wall. Even in the epithelial cells covering the villus a part at least of the fat is dissolved in the cell, the protoplasm of which allows it to be diffused readily on towards the basement membrane, but in the lateral membranes of these cells it may be retained in a dissolved state after the cytoplasm has got rid of its charge of fat.

We further know that in developing nerve-cells the chromatin of the nucleus diffuses out through the membrane of the nucleus to form the substance of the Nissl granules which are so distinctive a feature of nerve cells in adult vertebrates.[35] Further, in the developing ovarian ova of Amphibia the chromatin of the nucleus alters in composition, gathers in small spherules immediately adjacent to the wall of the nuclear membrane and gives off material which passes through the membrane to constitute the para-nucleoproteid (vitellin) of the yolk spherules.

A like diffusion occurs in the hæmatoblasts of Amphibia when the antecedent of hæmoglobin passes through the nuclear membrane to form hæmoglobin in the cytoplasm. We know from the observation of Sutherland Simpson and Hering[36] that hæmoglobin-like compounds occur in the cavities of hepatic cell-nuclei of the dog which were probably derived from red corpuscles, some of which were observed inside liver-cells either intact or in the process of degeneration. That hæmoglobin can diffuse into the liver-cells and into the nuclei follows from the observations of Browicz[37] who injected intravenously in a dog a quantity of a solution of Merck's hæmoglobin and found crystals of hæmoglobin in the hepatic nuclei. He observed also that when red corpuscles break down in liver-cells

hæmoglobin is stored in their nuclei but this can only be explained on the assumption that hæmoglobin diffuses through the nuclear membrane.

One may multiply the instances, but enough are detailed to show that membranes in, and the protoplasm of, the living cell are capable of allowing "colloids" to diffuse through them and that living animal membranes do not necessarily distinguish between colloids and crystalloids unless they are specially constituted for that purpose, and then, strange to say, they tend to be impermeable to crystalloids of the inorganic class.

One of the most striking results of my microchemical studies is the determination that the nucleus is absolutely free from chlorides and phosphates, and, as these form the typical salts of sodium, potassium, calcium, and magnesium in tissues and physiological fluids, it is obvious that these elements are absent also from the nucleus. Indeed, direct tests for potassium and calcium show unmistakably that salts of these elements do not occur in the normal nucleus.

It is a fact that in the intestine absorbing iron salts the nuclei of the epithelial cells whose cytoplasm gives a marked reaction for inorganic iron are wholly free from inorganic iron. In fact the whole cytoplasm may be surcharged with inorganic iron and yet not a trace is found in the nucleus. Even in the liver in pernicious anæmia when the cytoplasm of the hepatic cells is markedly impregnated with inorganic iron, their nuclei never show a trace of it.

From these and other observations I put forward the doctrine that the normal cell-nucleus does not know the inorganic world, that it is the home of certain organic compounds only.

Is this merely due to the greater avidity of the cytoplasm for inorganic salts and thus none of the latter are allowed to leave the cytoplasm for the nucleus, or does the nucleus offer of itself an obstacle to the penetration of it by inorganic salts?

The latter is, I believe, the correct answer. The head of the spermatozoon is an altered nucleus and in the seminal fluid apart from the spermatic elements there are the chlorides of sodium and potassium. Now if the cytoplasm is responsible

for the freedom of the nucleus from inorganic compounds the head of the spermatozoon should be charged with chlorides and yet not a trace of chlorides have I ever found in the heads.

It is manifest then that the nucleus actively excludes inorganic salts and the question is, How is it done? The answer is that the nuclear membrane is so constituted as to exclude them.

This impermeability of the nuclear membrane has in some cases been noted by others. Hamburger [38] found that when the intestinal epithelial cells are bathed with different concentrations of sodium chloride the cytoplasm is readily permeable to the salt but the nucleus manifests little or no permeability. This he found to be true also of the nuclei of the ciliated epithelial cells of the trachea and of the nuclei of the epithelial cells of the bladder.

The absence of certain other compounds, and these of the organic kind, must be specially noted. Except in rare cases, all pathological, no fats are demonstrated in the nucleus even with the most sensitive microchemical reagents for fat, like Scarlet Red or Sudan III. Further, intranuclear glycogen has never been observed in the case of normal cells and this of itself would postulate the absence of free sugar in the nucleus. We know also that free sugar has never been found microchemically in the nucleus. It is not quite certain, but it seems probable from observations on the point that the nucleus does not contain free proteids, like globulins and albumins.

The total exclusion not only of inorganic salts but also of fats and free carbohydrates from the nucleus and the probable absence of free proteins is a point of prime importance and it throws a new light on the relation of the nucleus to the cytoplasm.

What is found in the nucleus is chiefly an iron-holding nucleoproteid which the histologist calls chromatin and which differs for different kinds of cells, that of the pancreatic nuclei being somewhat unlike in character that of the hepatic nuclei and this again different from the nucleoproteid isolable from the nuclei of renal cells, although all are like one another in

the main in their composition. These iron-holding nucleoproteids are synthesized in the cytoplasm. Iwanoff [29] found that the zymase from yeast-cells synthesized phosphoric acid and sugar and formed a compound in which the sugar was masked and which did not give a reaction for phosphoric acid until it had undergone treatment for some time with strong nitric acid. This points strongly to the probability that the synthesis results in the formation of the skeleton of the nucleic acid molecule. As the yeast cell has no nucleus and as the zymase is a ferment or rather several ferments, it is very probable that nucleic acid is formed in the cytoplasm of the various cells of the body. The nucleic acid once formed readily combines with proteid and then the nucleoproteid diffuses out of the cell where it is synthesized, or into the adjacent nucleus where it is stored. Those which diffuse out may reach the interior of other nuclei or the nuclei of other species of cells.

The nuclear membrane is permeable to such iron-holding nucleoproteids. The diffusion outwards from the nucleus of the developing nerve-cell of all or nearly all its chromatin to constitute the material of the Nissl granules has been already referred to. The presence of prozomogen in the cytoplasms of secreting cells, such as those of the salivary, gastric, and pancreatic cells is due to a chromatin-like compound diffusing from their nuclei.

It is highly probable that the permeability of the nuclear membrane by the iron-holding nucleoproteids is due to a degree of solubility of the latter in the substance of the membrane, just as copper oleate is soluble in the membrane of Kahlenberg's experiments. Why the iron-holding nucleoproteids gather in the nucleus in such a quantity as we find there and do not diffuse out again is easily susceptible of an explanation. It probably passes through the membrane to the nuclear cavity in the water-solid phase, that is, in the hydrophilous form, but in the nuclear cavity it enters into the other condition, namely, the solid-water phase in which there is more solid and less water and consequently it is much less diffusible and more inert. There can be no doubt that the less diffusible phase does

occur inside the nucleus for it constitutes the chromatin masses, the chromatin network, and the chromatin loops so familiar to the cytologist and histologist. What transforms the one phase into the other can at present be only a matter of speculation, but it may be suggested that the nuclear membrane brings about this change, for the chromatin in not a few species of nuclei lines the inner face of the nuclear membrane, indicating that the transformation occurs in the membrane or in its immediate vicinity.

The nuclear membrane being permeable only or chiefly for iron-holding nucleoproteids two conclusions follow: one, that these compounds so long as they remain in the interior of the nucleus are protected from chemical alteration due to the action of inorganic and other compounds which may invade the cytoplasm; the other that to afford such protection is the true function of the nucleus.

These two conclusions based on the composition and the properties of the nuclear membrane enable us to comprehend clearly what constitutes the physical basis of heredity.

In Darwin's theory of heredity every cell of the body gives off typifying particles which he called gemmules and which collect in the ova and spermatozoa to reproduce in the offspring all the features of both parents.

This would account for the inheritance of immediately acquired characters, but the impossibility of such inheritance is now generally admitted. The main objection, however, to the theory is that it is impossible to conceive of such myriads of gemmules being accommodated in an ovum or spermatozoon.

The theory of heredity which has to-day received the widest assent is that of Weismann, which postulates that the inheritance of ancestral characters is due to transmission in the germ-cells from generation to generation of a substance, the carrier of heredity, called germ-plasma, which is not affected by metabolic and other changes in the somatic tissues of the individual but which may spontaneously vary and thus give rise to varieties in the offspring of the species. According to this theory no immediately acquired characters are transmitted to the offspring.

What provides for the protection of this germ-plasma which is handed down from generation to generation unchanged or changed only in the fashion that suggest the spontaneous origin of "sport" modifications of forms, Weismann does not explain.

It seems to me that the true explanation of heredity lies between the theory of Darwin and that of Weismann.

A germ-plasma in the sense implied by Weismann may exist, but on the view here advanced its continuity is one of type rather than of identical molecules, for the nuclear membranes of the germ-cells sort out or select from all the iron-holding nucleoproteids from the various portions of the body that reach such germ-cells those of a certain definite fixed composition and any other nucleoproteids that may be present are excluded from the nuclei of the ova and spermatid cells. Such selected or sorted out iron-holding nucleoproteids may in a manner represent the gemmules of Darwin's theory. Such compounds transmit the inherited parental characters and, to continue the transmission in the offspring of such characters, provide for the maintenance of some type of nuclear membrane in the germ-cells of the offspring.

Slight changes in the nuclear membrane of the germ-cells would provide for the variants, or "sports" in the offspring, but the nuclear membrane may itself be supposed to remain constant, although this constancy does not demand that the iron-holding nucleoproteids which pass through it are of an absolutely uniform type. Many isomers may be supposed to occur amongst these compounds and yet only a few of them may have the affinity for the material of the nuclear membrane which will enable them to pass through the latter. Miescher has pointed out that when an inorganic compound, *e.g.*, albumin, has at least 40 atoms of carbon, a billion isomers of it are possible, and this number may be greatly increased by variations in the position of the nitrogen atoms. How this may permit variations in the offspring while providing for transmission of the general characters need not be discussed here.

The nuclear membrane therefore makes the transmission of parental characters from generation to generation possible.

The nucleus, membrane, and contents, has thus been of immense service to organisms in the struggle for existence, for if the germ-plasma, the particular iron-holding nucleoproteid which is the all-important element in heredity, were freely exposed in the cell-protoplasm it would be subject to changes which the never-ending chemical processes in the cell would bring about, and it would also be affected by the salts which are constantly diffusing through the cytoplasm. That such salts would be harmful may be inferred from the fact that they do not occur in the nucleus. Any changes that would give rise to variations in the species must arise through the germ plasma being altered in composition but to such a slight extent that it would still be soluble in the nuclear membranes of the germinal cells and, in consequence, the offspring of the next generation would present variations. Such slight changes in the germ-plasma would be cumulative, would result eventually in a nuclear membrane constituted so as to permit only an altered germ-plasma derived from such cumulative action to enter the nuclear cavity and thus a new species with more or less fixed characters would originate. Without a nucleus, on the other hand, there would be no fixity of type or of characters. Whatever character one generation in a species would win that would be of advantage to it in the struggle for existence would not be continued unchanged in the next or it might not be transmitted at all. In this way chaos might result and there would be no progress or development such as there has been in evolution.

This constitutes the reason why the species of nucleated organisms vastly outnumber the non-nucleated. In the early history of life on the globe all organisms were non-nucleated but gradually some unicellular forms developed a nucleus which gave them an immense advantage in evolution and these throve and displaced the non-nucleated forms. Of the latter there now remain only the yeasts, bacteria and the blue-green algæ. The yeasts and bacteria have an external membrane which seems to have many but not all of the properties of a nuclear membrane and this explains their readiness to undergo altera-

tions from the condition of virulence to that of attenuation in cultures, in the animal body. Only in a parasitic or saprophytic life could such organisms continue their existence. The cells in the blue-green algæ are protected by two membranes exteriorly and they thus escape the results of the direct action of the salts of their environment, but their perpetuation, probably almost unaltered, from a very remote period in the history of the globe, while other organisms have not been so continued except in species which are the results of an ever-progressive evolution, shows what a handicap in the struggle for existence these two external membranes are.

After the development of the nucleus came the differentiation into sex, and this was an additional factor in promoting the function of heredity, for the union of two germ-plasms, as occurs in the fertilization of ova, simply doubles the certainty that the main ancestral features shall be transmitted to the offspring, and at the same time tends to communicate to all the individuals of a species character which may be of immense advantage to it in evolution in the long run.

From all that precedes it may be gathered how necessary it is to have a clear view of what osmosis fundamentally means from the purely physiological side, and it may be understood how through it the cell-nucleus, which knows neither fats nor free carbohydrates, serves as the sacred receptacle for the heredity-bearing substance, the germ-plasma. Through this conception of osmosis also it may be seen what an all-important part a simple physical property of matter plays and has played in the progressive evolution of living forms on our planet.

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THE FUNCTIONS OF THE LIVER IN RELATION TO THE METABOLISM OF FATS*

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THE liver is an organ with multifarious duties to perform. Of many of these it is possible to speak with some degree of scientific assurance. Something of its work in connection with carbohydrate storage has been known for sixty years. It has something to do with the removal of nitrogen from superfluous protein foods and with the manufacture of urea from this nitrogen. It takes its part in the transformation of purin bases, turning them out as uric acid or destroying them more completely still. It deals with the blood pigment that is past work and uses it to prepare coloring matter for the bile, the urine, and the fæces, and there are many other miscellanies in the chemistry of the body which have been put down to its activity.

But the fact that the fat absorbed from the food is specially diverted, unlike the other foodstuffs, from the stream of portal blood and conveyed by its own channels into the systemic circulation might be taken as an indication that the fats, at any rate, may be left for other organs and tissues to deal with. Fats are stored in the body in larger quantities than any other kind of food. But the storage places for fats are well known and the liver in mammals is not included among them.

Yet the examination of the amount of fat in the livers of men and other animals reveals very great variations, not only in the case of livers from the post-mortem rooms attached to hospitals for the sick, but in those removed from animals at the slaughter-house. A hard-and-fast distinction between these two

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classes of material it may not be possible to maintain, but anyone who has determined the amount of fat in the liver in a long series of animals that are presumably normal will have been struck by figures occasionally obtained that are very different from those that may be taken as normal average figures for this organ, different even from those that would be abnormally high for other organs. Taking one series of about forty animals, the livers of which I examined in this way, the great majority were found to yield between 2 and 4 per cent. of the higher fatty acids reckoned on the fresh weight of the tissue as the total amount present in all the different combinations of fatty acids the liver contains, but five out of the series, or 1 in 8, gave an analysis double or more than double this amount: a dog 7.8 per cent., a pig 8.3 per cent., and three sheep 7.3, 12.3, and 14.1 per cent. respectively. This last figure means that very nearly, if not quite, half the solid matter of the tissue was fat in some form or other. The same fact comes out in spite of the absence of extreme figures in some analyses carried out by Hartley at the Lister Institute. A series of nine analyses of the livers of guinea-pigs which had been fed on oats, cabbage, and hay showed the percentage of higher fatty acids reckoned on dry weight to range from 6.1 to 14.9. Another series of five analyses on guinea-pigs fed exclusively from the age of two weeks on bread, water, and cabbage gave variations from 6.7 to 12.6 per cent. A series of twelve rats that I examined gave a range of variation from 1.7 to 6.5 per cent. of the fresh tissue. In another series of rats examined in the same laboratory by the same methods there were fourteen, the livers of which contained less than 4.2 per cent. of higher fatty acids, the mean percentage being 3.1; while four others with more than 4.2 per cent. gave as the mean 7.2 per cent. I shall be referring later to some figures which I obtained from cats; five of these animals, to all appearance normal, gave the figures 2.2, 3.5, 3.9, 19.2, and 22.2 per cent. From the post-mortem room material may not infrequently be obtained in which still larger amounts than these are to be found; 60 or 70 per cent. of the dry tissue, and 23 per cent. or more of the fresh, may be isolated

as higher fatty acids after treatment with potash and alcohol and subsequent acidification.

A good deal of experimental work directed to the production of abnormal amounts of fat in the liver and other organs has been published by G. Rosenfeld, whose results are well known.[1] The method of estimating fat employed by him is one that is open to a number of criticisms. On the one hand, it must be admitted that it yields a larger weight of substance soluble in ether than other methods. But, on the other hand, it is impossible to compare the results obtained by this method even with one another, since there are many combinations of fatty acids which are soluble in ether: the free acids themselves, the neutral glycerides, the choline phosphoric acid combinations of the type of lecithin, jecorin, cuorin, and others as well. An ethereal solution may in one case be composed almost entirely of neutral glycerides; in another case more than half of it may be some phosphatide lipoid substance. Since the common component of all these compounds which is of greatest significance is the fatty acid radical, the most satisfactory method of estimating fats for most comparative purposes is to set the fatty acids free from all the combinations in which they occur, separate them from the other constituents, and weigh them. If the combinations from which they are obtained are simple neutral fats the yield will be about 95 or 96 per cent. of the fat. If they are lecithin it should be 70 per cent., whereas other phosphatides yield less than 50 per cent. The fat contained in the ethereal solutions of extracts of the heart or liver obtained by Rosenfeld's method do not commonly yield more than 60 per cent. of fatty acids, sometimes less, and this yield varies according to the composition of the extract. Nevertheless, the range of variations in the amount of the fat in the liver that Rosenfeld was able to produce experimentally was so much greater than in the case of other organs that such uncertainty as his method leaves is relatively insignificant. There is no organ that is ever flooded with fat in the way that the liver is.

Now the appearance of large amounts of a substance in an

organ may be due to active production of that substance in that organ or else to importation from other parts. We know from the famous experiments of Rothamsted, carried out by Lawes and Gilbert sixty years ago, that fats are synthesized by animals and that the material used for this synthesis is in the main at least the carbohydrates contained in our food. Magnus-Levy has described a reaction occurring in the liver which he regards as typical of the unknown reactions by which this synthesis is effected.[2] He found among the acids formed in the liver when portions of this organ were kept aseptically in a thermostat for some days, not only acetic and lactic acid, but also butyric acid, and at the same time gas was given off which proved to consist in part of hydrogen. The source of the butyric acid could not be determined positively, but there were some indications that it was derived from sugar and the simultaneous occurrence of hydrogen suggested an analogy with the butyric fermentation of sugar. And Magnus-Levy adopts as the explanation of the appearance of this butyric acid a hypothesis originally put forward by Nencki, which may be extended to account for the formation of higher fatty acids from carbohydrate. This is, that in the first instance lactic acid is formed from the sugar; that lactic acid, as is well known to be the case with all α-hydroxy acids,[3] readily splits into formic acid and the aldehyde with one less carbon atom than the hydroxy acid, in the case of lactic acid acetic aldehyde; that the aldehyde then condenses to form aldol, which is the β-oxybutyric aldehyde, from which it is easily conceivable that butyric acid may be formed. The condensation of aldehydes on this plan is not confined to acetic aldehyde, and either by the condensation of two molecules of aldol or four of acetic aldehyde it is equally conceivable that the same type of change as leads to the formation of butyric acid may lead to the formation of caprylic acid with eight carbon atoms, and so forth. The only limitation such a hypothesis imposes is that the acids produced must all contain some multiple of two carbon atoms, which is just what is well known to be the case with the fatty acids found in nature. If, therefore, butyric acid

is formed from glucose in the liver by such reactions as these the synthesis of palmitic and oleic and stearic acids from glucose may also be the work of the liver and be effected by similar reactions. In butyric fermentation of sugar, besides butyric acid, sufficient of the six-carbon caproic acids is produced to be commercially worth separating, and as Raper showed at the Lister Institute detectable and identifiable amounts also of the normal eight-carbon caprylic acid.[4] The reactions, therefore, which serve the bacteria for producing butyric acid from sugar serve them also for the synthesis of caproic acid and caprylic acid. Raper also succeeded in synthesizing normal caprylic acid from acetic aldehyde by condensation of aldol with itself, oxidizing the aldehyde so formed by means of silver oxide and reducing the hydroxyl groups in the chain with hydriodic acid,[5] an achievement which previous chemical experience as to the condensation of aldehydes resulting in the formation of branched and not of normal chains rendered improbable. The presence of hydroxyl groups in the chain seems to account for this. Magnus-Levy's observations, therefore, may be taken as pointing to the liver as a likely organ to which to assign the transformation of sugar into fat, since it has to be assigned to some organ in the body.

In a long series of experiments on the amount of higher fatty acids present in the liver I found that in a fair proportion of cases, nearly 50 per cent., this amount could be observed to increase when a pulp of the liver tissue was kept in a thermostat at the body temperature.[6] The weighed increase of fatty acids soluble in petroleum and not soluble in water from a sample of liver pulp weighing about 50 grammes was sometimes as much as 3.4 or even 5 decigrammes and amounted to a 15 to 30 per cent. increase on the fatty acids present in the pulp before the experiment started, as determined by reference to the mean between two control estimations, which differed from that mean by about 1 per cent. of the total amount of the fatty acids present. In three out of five attempts to remove and reduce the liver to pulp aseptically this was successful, and in two of these three there was a marked increase in fatty

acids on incubation, so that the formation of fatty acids was not the work of bacteria. As to the nature of the material which was converted into fatty acids under the conditions of the experiments, it was impossible to get clear evidence: the addition of substances which might be supposed to be used, or to occur in the course of the reactions involved, in no case gave constant and uniform results. The many negative experiments seemed to make it desirable to postpone further investigation till more light from other sources could be thrown upon the subject.

There are, therefore, grounds for looking to the liver as the seat of the synthesis of fatty acids, and it is possible that the reason why there are greater variations in the amount of fat in the liver than in other organs is in some measure to be sought for in this direction. It should be remembered, too, that the fatty acids found in the liver are to a much greater extent than is the case in the connective-tissue fat, unsaturated acids; and since the only hypothesis as to how fatty acids are synthesized for which there is anything to be said implies a possibility or even the probability that the acids formed would be unsaturated, this may be regarded as some further justification for the idea.

There are, however, still better grounds for thinking that large amounts of fat found in the liver are often due to the importation of fat from the storage places in the connective tissues. For in the first place, perhaps the most remarkable feature of the extreme cases of fatty liver described by Rosenfeld in dogs which were starved for five days and then given phlorhizin on the sixth and seventh days, is the extraordinary rapidity with which enormous quantities of fat appear in the liver, forming as much as 70 per cent. of the dry weight of the organ.[7] He says that the fat does not begin to accumulate until the fortieth hour after the first dose of phlorhizin, and the animal is killed eight hours later. According to this account, therefore, this enormous increase in the amount of fat in the liver takes place in the course of some eight hours. Again, if the animal is allowed to live another twenty-four hours, without

any further dose of phlorhizin the liver is then found to contain only normal amounts of fat, so that recovery is nearly as remarkably rapid as the development of this abnormal distribution of the fat in the body is. It would be easier to understand such rapid changes as these if it is not necessary to suppose the fat to have been made where it is found.

There are probably parallels to be found to the rapid changes indicated by Rosenfeld's results in clinical experience. Cases are recorded of the most intense fatty changes in the liver being found post-mortem when there is every reason to suppose that the abnormal condition has come on with the greatest rapidity. Cases of deferred chloroform poisoning particularly suggest themselves. Thus Langmead [8] describes the case of a boy, aged twelve years, who after operation under chloroform for lymphadenomatous glands in the neck became delirious and later drowsy and unconscious, and died, jaundiced and wasted, seventy-two hours after the operation with a very fatty liver; and another case of an apparently healthy child, aged three and a half years, dying thirty-six hours after a similar operation with similar sequelæ, and showing intense fatty change in the liver. He also describes a case where similar clinical symptoms followed a trifling operation under gas in an out-patient, a girl, aged fifteen years, accompanied as in the other cases by alarmingly rapid wasting. This case ended, however, in recovery, and ten days after the onset the body was restored to its normal condition of nutrition. Cases of cyclical vomiting ending fatally in three or four days are described with similar post-mortem appearances in the liver.

A case of a peculiar character has recently come to my notice through the kindness of Dr. A. E. Boycott, who sent me a portion of the liver for examination. A man, aged forty-nine years, apparently healthy, drank when intoxicated a considerable amount of strong hydrochloric acid and died six hours later. His stomach was practically destroyed. An adjacent portion of the liver was also burnt and this part under the microscope showed no more fat than is often to be seen in a normal liver. The rest was intensely fatty and contained

on analysis more than 20 per cent. of higher fatty acids reckoned on the fresh weight. The acids had the iodine value 65.6. Dr. Boycott's comment that "the presumption is pretty well justified that most of this fat had appeared in the liver in these six hours" is one with which I am strongly disposed to agree. Those who have a wider clinical and post-mortem room experience than I, may be able to furnish further evidence for the belief that I hold, that the most marked accumulations of fat in the liver are not necessarily by any means always slowly brought about by chronic disorders as in phthisis or chronic alcoholism. In many cases they may have arisen as rapidly, and in others perhaps are recovered from as rapidly as seems to be the case in phlorhizin poisoning in dogs.

It is unfortunate that the pathological examination of post-mortem room material is so largely confined to the histological and microscopical appearances. The estimation of the amount of fat in a tissue by histological reactions is a well-known source of fallacy. Hoppe-Seyler showed that there is less fat in a sciatic nerve that has degenerated as a result of section than there is in the healthy nerve on the other side of the same animal.[9] Rosenfeld proved the same to hold for a degenerated occipital lobe in man by comparison with the healthy lobe on the other side of the same brain. It is also familiar that whereas normal heart muscle gives no histological reaction for fat, it nevertheless contains something like 10 per cent., reckoned on dry weight, of higher fatty acids in different kinds of combinations, of which the microscope gives no indication. The hearts of animals, on the other hand, that give histologically the most intense Scharlach reactions for fat, on analysis may be found to contain relatively only a little more fat than normal hearts.[10] The chemical estimation of fat in tissues, when all appliances are at hand, hardly takes more time than the preparation of histological specimens, and it is urgently desirable that post-mortem room material should be systematically worked at on chemical lines as well as histological.

The classical controversy as to the source of the fat in fatty organs is, as a matter of fact, commonly regarded as closed.

Lebedeff twenty-five years ago made the two most important observations bearing on this point, and they have been confirmed by Rosenfeld using other methods. Lebedeff, in the first place, noted that the liver in a case of phosphorus poisoning supervening on extreme emaciation showed no fatty change. He suggested that the absence of the usual amount of fat found in the liver in phosphorus poisoning was due to the lack of fat in the rest of the body.[11] Rosenfeld has since administered phosphorus to dogs in different states of nutrition. He found in the liver of a well-nourished dog 37 per cent. of fat reckoned on dry weight; in a dog less well stocked with fat 17.4 per cent.; and in a dog reduced by starvation to extreme emaciation only 6.1 per cent., a low figure, that is to say, even for the liver of a normal animal.[12] Lebedeff's other observation was that if he substituted for the fat usually present in a dog's adipose tissue fat of another kind, linseed oil, the acids constituting which are liquid, and give lead soaps that dissolve in ether, then the administration of phosphorus causes the appearance of large amounts of fat in the liver similar in composition to the linseed oil contained in its adipose tissue, a fat, that is, yielding 67 per cent. of acids, the lead soaps of which dissolve in ether, whereas a dog not previously prepared when given phosphorus had in the fat in its liver only 23 per cent. of such acids giving soluble lead soaps. By means of the iodine value Rosenfeld has, as is well known, been able to show that the fat found in such large quantities in the liver in phlorhizin poisoning varies in its character with the character of the fat stocked in the animal's adipose tissue.

The most marked changes, therefore, in the amount of fat in the liver are due apparently to the migration of fat from the adipose tissues in which it is stored. For if it were produced in the affected organ there is no reason why the character of fat produced should be determined by the nature of fat in other parts of the body. And, as we have seen, the liver is the organ in which the most marked accumulations of fat are found. The liver is therefore the organ to which the migration of fat from other parts of the body is most conspicuous. In

other organs the amount of fat may be increased 30 or 50, possibly 100 per cent. above normal mean figures. In the liver the increase may be 300 or 400 per cent. above the normal mean, and even among normal animals something like 1 in 10, or even 5, give figures that are 50 to 100 per cent. or even more above those which prevail among all the rest. In fact there is in the extent and range of the variations in the amount of fat in the liver, as compared with other organs, something comparable to the range of variations in the amount of glycogen in this organ compared with others. But the interpretation of the appearance in the liver of the largest amount of fat ever found there so far seems to be different, in that the fat is, in the cases that have been most studied, imported from other parts of the body and not made on the spot.

Now there are important differences between the fat usually found in the liver or heart and that of adipose tissue. Substances soluble in ether extracted from the heart or liver by Rosenfeld's method are not entirely composed of fat in the ordinary sense of neutral glycerides of higher fatty acids: the yield of these acids on saponification is 30 to 40 per cent. too low. It is too low even for lecithin which is known to be present in these organs, and since some neutral glyceride is there too, there must be also some substances present, and not in negligible amounts, besides the simple glycerides and lecithin, either substances which contain less higher fatty acids than lecithin, such as jecorin or cuorin, or else others which contain none at all, such as cholesterin.

After having used, therefore, the Rosenfeld method of fat estimation for a time I made it a practice to check it by saponifying the extracts obtained and then determining the fatty acids. It is the fatty acids after all that are the essential factors in the metabolism of fat, and therefore for general biological purposes some method by which all the fatty acids, present in a tissue in whatever kind of combination, are set free and obtained in a weighable form is the most suitable and reliable. For some years now we have used some modification of the Liebermann method consisting essentially in dissolving

the tissue in strong potash and saponifying in the presence of alcohol all compounds of fatty acids, precipitating the acids with sulphuric acid, and extracting with petroleum boiling under 70° C. This method gives excellent results for most purposes. But it soon became obvious that the fatty acids obtained from the liver or heart were different from those in the fat of adipose tissue. When kept for a few days at the ordinary temperature, not protected from the air, or when heated for an hour or two, they increase in weight and are found to have become incompletely soluble in petroleum. Since the oxy-acids formed by oxidation of unsaturated acids are insoluble in petroleum this suggested an explanation of the behavior of the acids. And the fact that Rosenfeld's determinations of the power of absorbing iodine possessed by the extracts obtained from the liver and heart gave figures higher than those carried out on connective-tissue fat, in spite of the fact that they contained only about 60 per cent. of fatty acid, though it might be explained in other ways, might probably mean that the acids in the fatty substances he obtained were more unsaturated than those found in the adipose tissue. If the absorption of iodine by the extract was entirely due to unsaturated fatty acids, then in an extract absorbing 70 per cent. of iodine and containing only 60 per cent. of fatty acids, the fatty acids themselves must absorb about 116 per cent. of their weight of iodine. When Hartley therefore undertook a systematic investigation of the nature of the fatty acids in the liver and other organs this was the first point he attacked. By the use of methods calculated to expose the acids as little as possible to the risk of oxidation he found that the mixed fatty acids of the liver, heart, or kidneys from every species of animal examined absorbed far more iodine than the acids from the adipose tissue fat of the same animals treated in exactly the same way. The iodine value of the fatty acids in these organs averages normally about 120, and ranges from about 110 to 140, whereas that of the acids from adipose tissue varies according to the species from 40 in the goat to 65 in man.[15]

But the one organ, and this is the point that is of most importance in our argument, the one organ in which the iodine value of the fatty acids was found to present fairly commonly wide departures from this order of magnitude is the liver. In the following series of human livers examined by Hartley and Mavrogordato the more the amount of fat exceeds the normal average amount the lower the iodine value of the acids becomes, till in the cases of extreme fatty change the iodine value is no higher than that of the acids in subcutaneous fat. The list given is from their paper.[14]

TABLE I.

	Cause of death.	Higher fatty acids per cent. of dry weight.	Iodine value of fatty acids.
Normal figures	1. Pernicious anæmia	12.1	116.8
	2. Lobar pneumonia	13.7	116.8
	3. Pernicious anæmia	14.25	116.0
	4. Diabetes	14.4	119.6
Commencing fatty change	5. Toxæmic jaundice	15.6	109.5
	6. Accident	17.2	103.5
	7. Empyema	21.5	96.0
	8. Phthisis	25.4	96.4
	9. Bronchopneumonia	38.4	84.9
	10. Appendicitis	44.0	91.1
	11. Carcinoma of bladder	47.2	77.8
	12. Bronchopneumonia	54.6	71.8
	13. Ulcerative colitis	60.9	80.3
	14. Accident	66.3	63.0
	15. Dysentery	73.5	69.1

This is a striking series, but it could be extended by a number of analyses carried out since, many of them on animals, which fully confirm the fact which it brings out—namely, that the more fat there is in the liver the nearer this fat approaches in character to that of the fat stored in adipose tissue. It is difficult to see how this is to be explained except by supposing that when there is an excessive amount of fat in the liver it is because an excessive amount of the stored fat of the body has been brought to the liver. And the inference comes ready to

hand that a normal liver, too, gets fat brought to it composed largely of saturated acids but this fat it is able to deal with, converting the saturated acids into less saturated ones. We have not yet obtained sufficient figures for other organs, the heart or kidney for instance, to be able to say positively that variations of this character do not occur there too, but we certainly have not obtained, nor do we any longer expect to obtain, variations on this scale.

The liver, therefore, so far as we can see at present, seems to be the organ of all others to which the connective-tissue fat can most certainly be traced. And yet when this importation is not excessive the fatty acids in the liver are like those in the other working organs of the body in having a high iodine value, and in this respect unlike those in the stored fat of the connective tissues. This suggests that the fat which is imported to the liver is normally there altered so as to acquire a higher iodine value. Such a desaturation of fatty acids must occur somewhere, since the fat stored away and not in use is composed of acids that are more saturated than the fat in any of the organs in which we can suppose fat to be oxidized. It may be, of course, that desaturation is effected in all cells which oxidize fat, but the facts with regard to the liver that we have been reviewing make it probable that it is especially in the liver that this change is brought about, and it seems possible that the liver to a great extent prepares the fat in this way for other organs in which the oxidation is subsequently carried to a finish.

In order to see if it was possible to get any evidence of this desaturation of fatty acids in the liver I proposed last summer to Dr. L. Meyer Wedell some experiments in which animals were fed on various fats and oils. Her experiments were unfortunately interrupted, but not before one curious and astonishing fact had come to light. The fatty acids in the livers of rats have the iodine value of about 130, sometimes rather more. Cod-liver oil yielding acids with the iodine value of 150 was given to rats from four to fourteen days, and it was found that the iodine value of the fatty acids in their livers

went up; the figures obtained were 161, 198, 170, 173, and 174. Subsequently I repeated these experiments in this way: 5 grammes of ground rice, 4 grammes of plasmon, and 3 grammes of gelatin were heated together in 100 cubic centimetres of water, containing a little sodium chloride, calcium chloride, and potassium phosphate. While still warm this was well shaken with 30 cubic centimetres of cod-liver oil and a fine emulsion obtained. Then while still being shaken it was cooled down under the tap and just before setting poured out into

TABLE II.—*Showing Effect on the Liver Fat in Rats of Feeding with Cod Liver Oil.*

Rat.	Weight	Duration of feeding	Approximate amount of oil eaten.	Liver.		Skin.	Rest of body.
				Fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.
No.	Gm.			Per cent.			
1	150	0	0	1.7	135	84	108
2	154	<16 hours.	3 c.c. (much food in stomach).	2.3	131	100	112
3	200	*48* hours.	*20* c.c.	4.9	*215*	83	..
4	196	4 days.	7 c.c.	4.9	184	103	130
5	226	6 days.	21 c.c.	2.8	177	94	132
6	161	7 days.	14 c.c.	2.7	167	88	136

Iodine value of fatty acids from cod liver oil, 150.

a basin. By determining the amount of this emulsion in jelly that was eaten a rough idea of the amount of oil taken could be formed; the Tables II and III show the same sort of figures for the iodine value of the fatty acids in the liver, higher, that is, than any that ever have been observed under other conditions, *and even higher than those for the oil given.* The subcutaneous fat tends to be affected in the same sense, but to nothing like the same extent nor so quickly. In the case of the liver the effect is already well marked within twenty-four hours of the beginning of the feeding. The contrast between the rats that

had cod-liver oil and those that had none is constant, and is still more marked with those that had fat of a low iodine value, cocoa-butter. The other organs of a rat are too small to be satisfactorily worked in this way.

In order to get figures for other organs it is necessary to take a larger animal. Cats, which, of course, take fish greedily, were selected. It is well known that herring oil has a high

TABLE III.—*Showing Effect on the Liver Fat, as seen within 24 Hours, of Feeding with Different Fats or Oils.*

Rat.	Weight.	Amount of jelly eaten.	Fat or oil eaten.	Liver.		Skin.	Rest of body.
				Fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.
No.	Gm.	Gm.		Per cent.			
1	125	50	0	2.8	139	87	122
2	168	8	<1 gramme ol. morrh. (iodine value 150).	6.5	146	72	79
3	186	25	4.5 grammes ol. morrh.	4.8	173	75	86
4	237	30	5.5 grammes ol. morrh.	4.4	173	85	86
5	187	40	8 grammes margarine (iodine value 40).	4.8	133	71	76
6	203	25	5 grammes cocoa butter (iodine value 8).	5.1	113	74	80

The rats were kept without food for 24 hours, then each was given 50 grammes of jelly prepared with or without fat, and killed 24 hours later. All these rats were very well nourished and fat before the experiment began.

iodine value,—in the specimens which I examined 127. This oil forms about 8 per cent., according to my determinations, of the fat of the muscles of the fish. The most remarkable fatty acids, however, appear to be obtained from the ovary of this fish. The samples analyzed contained about 5 per cent. of fatty acids with the iodine value falling between 180 and 190.

No. I.—A cat was given three meals consisting respectively of 108 grammes and 210 grammes of herring roe and 90 grammes of herring flesh rubbed to a paste with 10 cubic centimetres of cod-liver oil. It was killed forty hours after the first meal.

No. II.—Another cat ate at its first meal 95 grammes of herring roe, at its second 130 grammes of herring flesh with about 12 cubic centimetres of cod-liver oil, and at its third 143 grammes of herring roe. It was killed sixty hours after the first meal.

No. III.—A cat fed on 100 grammes of cod flesh daily with no fat for four days, then given one meal of herring roe. After four hours the cat was killed, the contents of its stomach removed, and the amount of fat in this determined. The amount of fat in the food eaten was known, the difference gives the maximum amount of fat that can have been absorbed—namely, 2.3 grammes. The contents of the intestine were not collected or analyzed but certainly contained a part of this 2.3 grammes not yet absorbed.

No. IV.—A cat fed on 100 grammes cod flesh daily with no fat for five days, then given one meal of herring roe and killed eight hours later. The fat in the food eaten, less the fat in the food found still in the stomach, amounted to 3.3 grammes. Probably the amount actually absorbed was considerably less than this.

No. V.—A cat fed on 100 grammes cod flesh daily with no fat for five days, then given about 24 cubic centimetres cod-liver oil with its food in the course of forty hours and killed sixteen hours after the last meal.

Four normal cats, A and D, fed on horseflesh and milk were examined for comparison, and one, E, that had 100 grammes of cod flesh daily and no other food for five days.

The figures given in Table IV show that the liver is the only organ of those examined in which the result under consideration is noticeable. The oil finds its way, much the larger part of it no doubt, to the connective-tissue depots and tends to raise the iodine value of the fat already there with which

it mixes. The same thing happens in the liver, both in cats and rats, and, as the experiments with cats show, not on the same scale at any rate in any other organ; not at all, for instance,

TABLE IV.—*Showing Amount and Nature of Fats in the Organs of Normal Cats A to E, and Cats fed on Fish Oils I. to V.*

—	Liver.		Heart.		Spleen.		Kidneys.		Adipose tissue.
	Fatty acids.	Iodine value of fatty acids.	Fatty acids.	Iodine value of fatty acids.	Fatty acids.	Iodine value of fatty acids.	Fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.
	Per cent.		Per cent.		Per cent.		Per cent.		
A.	2.54	137.7	2.26	125.4	1.96	103.9	4.3	73.1 73.0	69.7
B.	2.22	129.4	2.99	127.8			6.0	61.1 61.2	
C.	3.95	136.9	3.06	119.1			4.6	72.8	73.5
D.	22.25	94.5	3.01	102.2			6.8	66.7	61.25
E. (Fed on cod flesh for 5 days.)	19.2	90.6	2.55	118.5	1.93	116.5	5.4	77.4	59.1
I. Herring roe 40 hrs.	2.96	181.7	3.58	116.4	2.31	140.0	3.7	100.3	94.8
II. Herring roe 60 hrs.	5.74	170.7	3.00	120.7	2.27	121.6			88.2
III. Herring roe 4 hrs.	2.58	152.5	3.08	112.0	1.95	118.0	3.97	80.5	67.5
IV. Herring roe 8 hrs.	3.50	166.9	2.33	137.9	2.62	106.0			84.6
V. 24 c.c. ol. morrh. in 40 hrs.	4.04	*185.0*	2.21	130.3					73.8

in the heart, an organ in which everything tends to show that fat is used as a source of energy very largely. And in the liver the effect is much more marked than in the adipose tissue

because the liver is not a place to which fat comes to stay, or in which it is stored. The character of the fat in the liver is determined not necessarily by the character of the fat which has been eaten and stored by the animals in weeks past, but by the character of the fat that has recently been circulating in the blood. This, of course, may in times of mobilization be fat from the stores in the adipose tissue. But when there is abundant fat taken up from the intestine mobilization of stored fat does not occur; the animal does not get thinner but fatter if plentifully fed. In this case the liver takes up the food fat and in the liver this food fat is more obvious than in the connective tissues, because in the liver it is not diluted down with fat eaten, it may be, weeks or months ago, since fat is not stored in the liver, but disappears from it to make place for other fat that it can get from the blood in its stead. The character of the fat in the liver, therefore, is determined in the first instance by the character of the fat offered it by the blood.

But in the liver there is something more than this. If this were all that happened the iodine value of the acids might approximate to that of the cod-liver oil acids but could not exceed it. In all the rats it exceeds it and also in Cat No. V., which is the only cat that is strictly comparable. This can only mean that the liver has the power of desaturating fatty acids and exerts it even when the acids supplied are already as unsaturated as those of cod-liver oil. Why even in this case the change is brought about we can at present only speculate. We know that the properties of unsaturated fatty acids of the same empirical formula vary according to the position of the unsaturated linkage. For instance, the ordinary oleic acid has the double bond in the middle between the ninth and tenth carbon atoms. Le Sueur prepared synthetically the acid with the double bond between the sixteenth and seventeenth, or, to use the ordinary nomenclature, between the α and β carbon atoms. This acid would not decolorize bromine water.[15] In certain positions, then, the double linkages are more stable than in others. It may therefore be that the liver is in the habit of removing hydrogen and introducing double bonds in certain

positions in the chain and does so whether there are such unions in other positions or no. If the acids of the fish oils are unsaturated in the wrong places they might then become still more unsaturated when brought within the sphere of action of the liver. On the other hand, it may be that there are double unions in the acids of cod-liver oil which do not absorb iodine as readily as they would in other positions in the chain—are not, that is, so liable to oxidation. The action of the liver may be to move these double linkages along the chain into positions in which they not only absorb iodine more readily, but are more liable to the disruptive oxidizing action of the cells that have to depend on fat for the supply of energy. The one idea involves the formation of new double bonds, the other a transposition of existing ones.

However that may be, the conclusions which are indicated by the observations described are, in the first place, that the liver has after all something to do with the fat absorbed from the intestine. Its position gives it, it is true, no special privileges in this respect. The absorbed fat being conveyed into the systemic circulation, other parts of the body have an equal chance of taking it up as it is brought to them in the arterial blood. So far as we know at present of these other parts of the body it is only the connective tissues that avail themselves of the opportunity. And they take the fat up as it comes to them and store it unchanged. The liver, on the other hand, not only takes up its quota, but gets to work on it at once, and brings about changes in the acids, for which the term "desaturation" may be convenient, though it must be remembered that sometimes possibly the change may be merely a transposition of unsaturated linkages, while in other cases the process must apparently be of the kind which the term desaturation implies.

I think it will be admitted that support is thus gained for the idea that the liver has work to do in preparing fat for the metabolic processes in which fat is concerned. There are no *a priori* grounds for thinking that this work is more than of a preparatory nature; the complete oxidation of fat liberates so much energy that the later intensely exothermic stages of

the breakdown of fat are likely to be reserved for organs where the discharge of energy is conspicuous—the heart, for instance, or the muscles or kidneys. If there is an extensive discharge of energy in the liver it is curious that it is so difficult to say what form that discharged energy takes.

It looks as if the work of the liver consisted in an operation which may be compared to the drying of gunpowder. The fats we take in our food are remarkably unreactive substances, and it has always been one of the most astonishing chemical achievements of animal cells that they should be able to burn up completely and cleanly as they do so stable a structure as saturated fatty acids like palmitic or stearic acids. They are wet gunpowder. And the body stores its gunpowder wet, and safely removed from the inflammatory operations of busily working cells. When the orders for mobilization are issued this wet powder is conveyed to the drying chambers in the liver, and from there distributed to the fighting line in a proper condition for use. There are times when the stress of this work is manifestly too great. Too active a mobilization of stored fat, or too little activity in dealing with it on the part of the liver, will result in an accumulation of the unfinished product in that organ. A fatty liver is then the result, and the fatty acids which it contains are found to have a low iodine value. There is an accumulation of wet powder.

Some of the conditions in which such accumulations are found appear to fit in with this conception of the way in which they come about. Pflüger, in his account of an important experiment on pancreatic glycosuria, noted that the dog which had excreted more than three kilogrammes of sugar in the course of two months, during which it was given no fat or carbohydrate, was at the end emaciated to such a degree that no fat could be found stored anywhere in its body. Nevertheless, its liver had not wasted and contained a normal amount of fat, 2.7 per cent. Hence he concluded that the liver keeps itself in a state of nutrition in diabetes, as the heart does in all forms of starvation, because of its activity which consists according to him largely in converting into sugar the fat

brought to it from the storage places for fat. So far as the transportation of fat is concerned we must, I feel, be with Pflüger; as to the way in which fat is worked up in the liver, Pflüger's position is one that is at any rate questionable. The fatty livers that occur not uncommonly in diabetes and the condition described by Rosenfeld in dogs poisoned by phlorhizin would be intelligible if interpreted as the result of excessive activity in the mobilization of stored fat due to the necessity in which the body finds itself owing to the draining away of so much carbohydrate. The mobilization of fat must, of course, occur always in starvation. Mottram has shown that when this occurs the amount of fat in the liver is increased. A preliminary note by him on this subject [14] appeared eighteen months ago. His proof since then has become far more circumstantial and convincing, and though this valuable work is not yet published I have his permission to refer to it here. He experimented by feeding rabbits and guinea-pigs for a long period on a constant liberal diet so as to have a number of animals in as nearly the same condition of nutrition as possible. Some were killed to serve as normal controls and others after one, two, three, four, or five days' starvation. Evidence was obtained in this way that an importation of stored fat to the liver occurs after one day and again after four days' starvation. At these periods not only is the amount of fat in the liver increased, absolutely as well as histologically, but the iodine value of the fatty acids is lowered. In the intervals the importation ceases, the liver being at work on that which has been brought to it, the amount of fat in it diminishing and the iodine value rising.

It is not, of course, to be expected that active mobilization of fat should necessarily and in all cases outrun the powers of the liver for working up and handing on the fat to other parts. A tendency for the liver to be in arrears of work is all the evidence we can expect from the examination of the organs of starving animals. Just as in the matter of alimentary glycosuria, we must expect to find individual differences.

On the grounds, therefore, which I have given I think it is

a fair hypothesis to make the starting point for work that the liver stands in a different relation to circulating fat from the other organs of the body. Its work is to take up this fat and bring about certain changes in it, the result of which is to make this material available for the use of the organs in which its potential energy is required. I have described the grounds we have for thinking that this change consists, at any rate in part, of a desaturation of the fatty acids. Is that the whole of the change? There is another difference between what may be called organized fat—the fat, that is, in the cells of the heart, muscles, kidneys, etc.—and the unorganized merely stored fat of the adipose tissue. The latter is the unaltered fat of the individual's food; it has its iodine value determined by that of the fat which has been absorbed, but it also contains no phosphorus. The fat in the liver and the other organs of the body, the organized fat, is very largely composed of phosphatide lipoid substances. Of these we know more or less dimly of a considerable number, more or less definitely of one, lecithin. Rubow estimates roughly that about a half, or sometimes more than a half, of the organized fat is "lecithin."[17] It has also been repeatedly noted that the fatty acids which can be obtained by saponification of lecithin or other phosphatide lipoid substances are more unsaturated than those from the adipose tissue.

It may therefore be that the work of the liver consists in the conversion of food fat into organized fat and that the desaturation of fatty acids is a change which occurs after that conversion has taken place; a change that is, therefore, removed from our ken so long as our knowledge of the nature and constitution of these lipoid molecules is as meagre as it is at present. The change may be brought about behind a veil within the mysterious circle of influence of the phosphoric acid and the nitrogenous basic constituents of these more complex molecules. In order to ascertain whether this is so Kennaway examined the acids of a number of extracts which I had prepared in fractions from normal and pathological livers.[18] The tissue was extracted repeatedly with alcohol, and then repeatedly with ether, in the cold. The solvents were evaporated off completely at a

low temperature and pressure and the residue taken up in dry ether and filtered. The ethereal solution was treated with acetone, which, broadly speaking, precipitates phosphatides but dissolves simple glycerides or ordinary fats. The precipitate contains nitrogen and phosphorus in about the amounts found for the best characterized lipoid complexes or mixtures of these; the acetone solution contains these elements in about one-tenth of those amounts. The precipitated substances when saponified yield insoluble higher fatty acids, soluble in light petroleum, in amounts which fall within the limits of the amounts in known lipoids, less, at any rate, than 70 per cent. The unprecipitated substances contain amounts of these acids which approximate to those found in pure simple glycerides more or less closely—that is to say, to about 95 per cent. But the iodine value of the fatty acids obtained from the simple glycerides was invariably higher, and generally considerably higher, than that of the acids in adipose tissue fat. It was lower, it is true, than that of the acids in the lecithin fraction of the acetone precipitate, the fraction, that is, which is soluble in absolute alcohol, and perhaps still lower than that of the acids of the jecorine fraction, which is insoluble in alcohol as well as in acetone. It would seem, therefore, that even the simple glycerides in the liver are largely compounds of acids which have undergone the desaturation process; in so far as they are not, they consist of the raw material not yet dealt with in this way. The reaction involved, therefore, is not one that is carried out only in the lipoid molecules of which we know as yet so little but is comparatively accessible, and there is fair ground for hope that further work on the subject may bring to light much more that is definite with regard to it.

The same sort of change clearly has been carried out in the case of the fatty acids found in the liver of fish like the cod, which yield an oil with a high iodine value. A most interesting investigation into the nature of the acids contained in cod-liver oil has been carried out by Bull. He prepared the methyl esters of the fatty acids and distilled these in vacuo fractionally. From the different fractions he isolated, in addi-

tion to palmitic, stearic, and oleic acids, myristic, the saturated acid with fourteen carbon atoms, and three other acids of the oleic series, one with sixteen carbon atoms, clupeoleic, corresponding to palmitic in the saturated series, one with twenty, called gadoleic, and an erucic acid with twenty-two carbon atoms.

None of the acids isolated, however, account for the high iodine value of the mixed acids obtained by saponifying this oil, 150 or more. The isolation and characterization of acids with more than one double link is a matter as yet of considerable difficulty. The most definite results have been obtained so far by oxidizing the mixed acids with alkaline permanganate in the cold. Saturated acids are not attacked, unsaturated acids become saturated by the assumption of hydroxyl groups. Oleic acid thus becomes dihydroxystearic, lineolic becomes tetrahydroxystearic acid, and so forth. These hydroxy acids can be separated, purified, and identified, though the yields are not always very satisfactory. The further oxidation of these hydroxy acids on being heated with permanganate results in the cleavage of the acids into two parts, at the position of the original double bond. This is one of the ways in which ordinary oleic acid has been shown to have its double link exactly in the middle; for the two acids produced each contained nine carbon atoms, one being a di- the other a mono-carboxylic acid; thus

$$C_8H_{17}.CH = CH.C_7H_{14}.COOH$$
Oleic acid.

$$C_8H_{17}.COOH + COOH.C_7H_{14}.COOH$$
Pelargonic acid + azelaic acid.

Another way of studying the unsaturated acids that has been much used is to saturate them wth bromine and examine the products. The more bromine these contain, the more limited is the solubility in ordinary solvents.

Hartley has for some time been engaged on the study of the fatty acids present in various compounds in the liver of

the pig. The results will very shortly be published in full, but some of the more striking results of his work may be alluded to here. On oxidizing with permanganate in the cold a dihydroxystearic acid is obtained which has a different melting point from that of the dihydroxystearic acid obtained from ordinary oleic acid or from the pig's adipose tissue fat. On further oxidation this acid breaks up but does not yield pelargonic and azelaic acid as the oleic acid in, for instance, the omental fat of pigs does. Hartley obtained pelargonic acid from this latter, but with the same treatment of the dihydroxystearic acid from the pig's liver he obtained again and again no volatile fatty acid but caproic. This seems to imply that the double bond in the hepatic oleic acid is in a different situation, not in the middle but one-third of the way down the chain starting from the unoxidized end. This, then, would be an instance of the transposition of the double bond to which reference was made earlier in the lecture.

The constitution of none of the linoleic acids that occur in nature, the eighteen carbon acids with two pairs of unsaturated carbon atoms, is known. It is not known, that is to say, where the double links occur; there is obviously a large number of conceivable situations in which they might exist. Hartley has obtained from the fatty acids of the pig's liver one tetrahydroxystearic acid which melts sharply at a temperature a little above that given for sativic acid, the tetrahydroxystearic acid obtained from vegetable oils, and which on analysis gives figures agreeing exactly with the formula $C_{18}H_{36}O_6$. He has obtained also a certain amount of evidence indicating the presence of a second tetrahydroxystearic acid in the oxidation products of the liver fatty acids. This latter is more soluble in boiling water and has a lower melting point than the former. If possible it is hoped to identify the products of further oxidation of these acids derived from the linoleic acids in the pig's liver. It is these linoleic acids that seem to be the most abundant of the unsaturated acids in this organ and to be principally responsible for the high iodine value of the hepatic fatty acids.

But among the hydroxy acids which Hartley has obtained is a curiosity; one that from its solubility in water must evidently contain a large number of hydroxyl groups. It crystallizes well, and melts sharply, and on analysis gives figures agreeing exactly with those for octohydroxyarachidic acid. This indicates the presence of an acid in the liver which has a chain of twenty carbon atoms and four double links. Further evidence of the occurrence of this acid he obtained in the formation of its octobromide on treating the acids with bromine. Anyone who has estimated the iodine value of the fatty acids from liver fats or oils must have noticed a precipitate that is formed by the iodine solution on reacting with the fatty acids. In the case of the acids from the pig's liver this precipitate appears to contain no hexabromide and to be composed entirely of this octobromide. This substance is insoluble in every solvent that has been tried, and is isolated only by extracting the bromination products successively with ether and benzine. This, of course, is not a very satisfactory way of arriving at a pure product. But several distinct preparations of the insoluble substance left have analyzed correctly for octobromarachidic acid, and, taken together with the analysis of the oxidation product mentioned above, this seems to be fairly satisfactory evidence for the occurrence of this remarkable acid, $C_{20}H_{32}O_2$. Hartley estimates that it is present in fair amounts, and constitutes perhaps 8 to 10 per cent. of the acids in the pig's liver. An acid of the same formula is thought by Bull to be present in herring oil, whereas Tsujimoto obtained from this oil and from Japanese sardine oil an acid with eighteen carbon atoms and four unsaturated links.

There are, indeed, clearly, yet things to be learnt with regard to the fatty acids of the liver. But a beginning has been made and indications exist as to the nature of the biological significance of some of the facts that have already been determined. To assign its place in the metabolism of fats to this remarkable acid, however, is a task that we are not as yet equipped to undertake. It may be a product of that synthetic activity of which there is some evidence in the liver; but it is



impossible to say. Small traces of arachidic acid occur in butter, and the corresponding alcohol, with twenty carbon atoms, has been found in the fat of dermoid cysts.

The more one works at the subjects on which I have had the privilege of addressing you this evening the more he becomes aware of the difficulties and obscurities that confront him. In view of these the few facts I have been able to muster present but a thin array which I have in vain tried to stiffen with thinner speculations. But a fact or two and a few changes of hypothesis constitute the journeyman investigator's stock in trade. And I have laid mine before you in the hope that you may be interested in them and in what I hope they may lead to.

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SOME PROBLEMS IN IMMUNITY AND THE TREATMENT OF INFECTIOUS DISEASES *

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SOME FUNDAMENTAL FACTORS OF PATHOGENICITY AND INFECTIOUSNESS.

IF the animal body and the bacteria were absolutely fixed in their physiologic character, varying in these neither qualitatively nor quantitatively in the adaptive sense when brought into contact with each other, it would probably be a much simpler matter than it is, to determine by careful study and experiment the exact factors underlying the conditions of pathogenicity and infectiousness of micro-organisms, and the reasons for the weakness or strength of the body when invaded by them, and we might thus, from a knowledge of the physiology of any given animal and bacterium, prognosticate the result of their activities, the one on the other. While this, of course, is not the case, still there are certain more or less fundamental requirements which experience has taught us must be met by an organism to be infectious for any given animal, and by infectious I mean the ability of an organism to live and multiply in the animal fluids or tissues. For instance, an organism which is shown not to grow at the body temperature of warm-blooded animals may safely be assumed not to be infectious for such animals; and experience is gradually teaching us that strictly aerobic organisms, those thriving only in the presence of free oxygen and not able to obtain this gas in available combinations from carbohydrates, can also safely be excluded from the infec-

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tious class. We have also learned that anaërobic organisms, although infectious when gaining entrance to tissues not abundantly supplied with blood, are practically unable to multiply in the blood stream and give rise to generalized infections.

There are a few facts such as these which once in our possession, gained from a study of the organism apart from the animal body, form the basis for opinions as to the entire exclusion of organisms from the infectious class or as to the restriction of these organisms to certain body regions during the life of the infected animal, and which give us by simple cultural experiments and knowledge of the effect of physical environment an idea as to the possible infectiousness or non-infectiousness of the organisms under consideration.

Most of the other factors determining infectiousness or the distribution of organisms in the animal body must be worked out in each case by actual animal experiments, and even after years of experimentation many of these factors are little known, for researches have demonstrated that bacteria showing little or no difference in their physical and chemical requirements under cultivation differ widely in their effects on the same animal economy, and that even the same organism may vary from time to time in its effects on the same species of animal; so that it is obvious that pathogenicity or even infectiousness is not an inconditionate character of all germs or even of those known to have been pathogenic.

FACTORS OF AGGRESSION AND DEFENCE OF THE MICRO-ORGANISMS.

It is my desire to focus attention for a while on some of the facts which have been worked out in regard to the chief factors of aggression and defence of the micro-organisms, laying aside for the time being the special animal body in which these may be active or, indeed, alone called forth, and assuming that the organisms possess all of the obviously requisite characters for the rôle of infectious germs.

In the first place, I think, it should be made clear that infectiousness and pathogenicity are not synonymous terms. An organism may be exquisitely infectious—or, possibly better,

parasitic—for a given animal, and yet its pathogenic effects may be of extremely slow development or almost *nil*, while another germ may be extremely pathogenic and yet little adapted for the life of infectiousness or parasitism; the very fact of its non-adaptation may apparently, in some instances, be the cause of its pathogenic action. Other organisms may have both requisites for the struggle with the opposing animal forces.

To understand more fully this point of view, it is necessary briefly to recall certain facts which are known about the physiology, metabolism, and composition of the bacteria, and of their ability to neutralize directly or to respond adaptively to the agents directed against them by the invaded animal. Some of these facts are so well known that passing mention alone is sufficient: such, for instance, is the fact that certain microorganisms, especially the bacilli of diphtheria and tetanus, secrete soluble poisons both during artificial cultivation and during their life in the animal body, which poisons are eminently toxic. These poisons are true secretions and are largely independent of the composition of the surrounding medium so long as this favors the physiologic activities and growth of the germs. Such germs, then, once having gained even an insecure foothold in the animal body, by no matter what favoring circumstances, are possessed of a powerful weapon of offence against the sensitive physiologic bases of the host, and of defence against its more immediate and mobile means of combating the germs themselves. In the case, however, of most other pathogenic bacteria, the secretion, at least in artificial media, of such highly soluble and potent poisons has not been demonstrated satisfactorily, although certain investigations point fairly conclusively to the production of some minor bodies which have been shown to act deleteriously on the red blood-cells and on the leucocytes—the hæmolytic, leucocidic and leucolytic substances which are looked on as probably true soluble toxins, like the toxins of diphtheria and tetanus. Other investigations support the idea that there may be still other true toxins secreted by these organisms, which also give rise in the animal body to the production of true antitoxins; *i.e.*, which are found

to be neutralized by their antisera, unit for unit, according to the law of multiples, just as are diphtheria and tetanus toxins.

Other minor poisons may in some instances be demonstrated in culture media, and also may possibly be formed in the animal body by the metabolic activities of the germs. These are either simply waste products of metabolism or bodies due to the decomposition of the nutrient media in which the germs are growing. These bodies are usually referred to as ptomaines, and differ entirely from the true secreted toxins, both in their chemical composition and in their physiologic action, resembling in both of these the alkaloids. They are not known to give rise to antibodies of any kind in animals. Apart from all the poisons just mentioned, *i.e.*, the toxins, hæmolysins, leucocidins and ptomaines, there is supposed to exist a most vitally important and interesting group of poisonous substances, the so-called endotoxins. These, so far as our knowledge goes, are poisons rather firmly seated in the bacterial cell, which are not secreted in our ordinary cultural media, and are supposed by most observers not to be separable in the animal fluids and tissues from the intact bacterial cell. These poisons may be demonstrated in old cultures, in which the bacteria are dead and disintegrating or undergoing autolysis—although Pfeiffer does not consider autolytic products necessarily similar to endotoxins—or they may be obtained by destroying the bacteria mechanically by pressure and grinding, or by breaking them while frozen. In the animal body they are said to become free when the bacteria die and decompose or are disintegrated by the digestive bodies by which they have been attacked. These endotoxins are recognized by the fact that they do not call out true antitoxins which become free in the plasma and serum, but do, nevertheless, lead to the formation of digestive antibodies, these not following, however, the "law of multiples" in protecting infected animals from the poisons. The liberation of these poisons by the destruction of bacteria in the animal body is best illustrated by the so-called phenomenon of Pfeiffer which takes place when cholera vibrios and immune cholera serum are introduced into the peritoneal cavity of a

guinea-pig. If specimens are withdrawn from time to time from the peritoneal cavity of an animal so treated, a rapid swelling up, disintegration and disappearance of the vibrios can readily be demonstrated. The organisms apparently do not multiply in the animal body under these conditions and are almost immediately destroyed. This disintegrating power is also claimed for the body fluids of normal animals and is supposed to be demonstrated by the following experiment. When graded quantities of a fresh cholera culture are introduced into the peritoneal cavity of normal guinea-pigs of equal weight, the following phenomena can be regularly observed: Minimal doses of the culture produce a febrile condition which continues for a few hours with no serious symptoms. Slightly larger doses give rise, after a short interval, during which there is fever, to a marked drop in temperature and definite symptoms of cholera poisoning—muscular weakness, twitching and general prostration. These symptoms of poisoning then gradually disappear, and after twenty-four hours the guinea-pigs are again normal. If the quantity of cholera culture injected is carefully increased up to the minimal lethal dose, the animal dies with all the symptoms of cholera intoxication, but on autopsy the peritoneum is found to be entirely sterile, or only a few isolated cholera spirilla are found, usually inclosed in pus cells. Finally, if larger quantities of living cholera spirilla are injected, the peritoneal cavity shows a profuse, serous, sometimes hemorrhagic exudate, which contains innumerable actively motile micro-organisms. The point of interest in this experiment is the demonstration of the fact that the normal guinea-pigs which receive enough of the cholera vibrios to prove fatal have destroyed the vibrios and presumably died from the poison thus liberated, and not from poisons secreted by living vibrios, or from an overcoming of their systems by the rapid multiplication of the organisms. It is only when the animal system is previously flooded with an overwhelming dose that the vibrios are found alive and multiplying even locally in the peritoneum after death. This does not mean, however, that no multiplication ever goes on hand in hand with

the destruction of the germs in the infected animal; on the contrary, such a multiplication is probably the rule rather than the exception, as has been shown fairly conclusively by the experiments of Radziewsky, and was beautifully illustrated by an experiment of Pfeiffer and Wassermann, who after having shown that the blood serum of human beings who have recovered from Asiatic cholera has the power to protect guinea-pigs from ordinarily fatal doses of cholera spirilla, even when used in high dilutions, then proved that this protective power is not an antitoxic one, but depends largely, if not entirely, on the ability of the serum to aid in the immediate dissolution of the vibrios. Thus animals which received only a fraction of a milligramme of such a serum were able to bear the injection of a loopful of virulent cholera vibrios, practically without reaction, while control animals succumbed to one-fourth of the dose with typical symptoms. Now, however, if the dose was increased to three or five loopfuls, not even ten thousand times the original amount of the serum would protect the animals against the inoculation. The toxic effects may, in fact, as shown by Pfeiffer, appear with extraordinary rapidity, so that in these animals the temperature may show the lethal drop within two hours after inoculation, while control animals which have received the same quantity of cholera germs without the serum may not show a similar lethal drop in temperature for four to five hours.

An explanation of the results of this serum is found, probably, in the fact that guinea-pigs are able to withstand a certain quantity of the intracellular cholera poison (endotoxin) which may be represented by one loopful of a fresh culture. If the animals are given smaller quantities without the serum, say one-fourth to one-half loopful, these may increase for a time without producing marked symptoms. Parallel with the increase, however, the phenomenon of germ destruction is going on and characteristic symptoms of intoxication appear at the moment when the number of vibrios destroyed has become so large that it corresponds to more than one loopful of the cholera culture. An animal will thus withstand a culture of

any size when mixed with immune serum, if the dose does not exceed the limit of intoxication before it is entirely destroyed. On the other hand, when guinea-pigs receive the larger dose of three to five loopfuls, the serum, not being antitoxic, is not able to counteract the fatal effects of the liberated cholera poisons, but, on the other hand, enormously increases the rate of destruction of the vibrios, and hence intoxication appears earlier in such treated animals than in the controls receiving the organisms alone.

I have selected this classic cholera experiment because I feel that it illustrates the most extreme limit of the endotoxin point of view, and, further, because the cholera organism, standing at one end of the scale, is the most extreme example of pathogenicity by virtue of its own destruction, while the diphtheria bacillus at the other end is one of the classic examples of pathogenicity by virtue of secreted toxins. Neither of these organisms is truly invasive or highly parasitic, and both are harmful usually by the action of their poisons alone and acting, as it were, from a base of supply on the periphery of the animal system. Between these two extremes stand all grades of infective germs.

These two organisms are typical examples of their kind, but there are few organisms which secrete such highly toxic soluble bodies as do diphtheria bacilli, and there are few so susceptible as the cholera organism to disintegration within the animal body; and yet there are many germs which are extremely pathogenic, and in many cases capable of severely and detrimentally infecting the animal body. In view of this unquestioned fact, I am of the opinion that the teaching which considers all poisonings as due either to true soluble secreted poisons, or to true endotoxins liberated only on disintegration of the bacterial cell, is too narrow; and it would seem probable that many organisms, possibly all, secrete bodies which are not soluble in their condition at secretion in culture media or in the body fluids, but which are susceptible to digestion in the animal body, and may thus become soluble and assimiliable, and when toxic act harmfully on the body cells. This question is an important one and

I shall speak of it later. Besides these actively poisonous bodies which we have been considering, there are probably bodies such as some at least of the bodies called aggressins by Bail, which, while not being toxic in themselves for the animal body, nevertheless are active defensive agents of the bacteria, probably neutralizing certain bodies of the animal economy, which are indirectly injurious to the bacteria. Further than this, certain bacteria may be furnished with envelopes, capable possibly of protecting them either chemically or physically from harmful influences.

In thinking over these questions I have gained some clarity of conception, based on visual perception by comparing some of the products of pathogenic bacteria with bacterial pigments and with insoluble interstitial or intercellular substance, which may be seen accompanying bacteria in cover-glass preparations. Soluble toxic secretions are to be compared to such pigments as the pyocyanin of *Bacillus pyocyaneus*, which is so readily soluble in culture media; endotoxins proper to pigments confined to the bacterial cell, or at least when secreted, being insoluble in culture media, such, for instance, as the well-known red pigment of *Bacillus prodigiosus*, which may often be seen free among the bacteria in irregular red granules like carmine powder. That bodies such as this latter might be extruded from pathogenic bacteria, and not be soluble in the usual culture fluids, is not improbable, and the fact that more or less insoluble interstitial substances are not infrequent among bacteria is well known. Among pathogenic germs these characters, in my experience, are usually, if not always, more marked in freshly isolated cultures. The sticky, almost slimy character of cultures of meningococcus may be recalled, a character which tends to disappear after a few generations of artificial cultivation, and the highly mucinous capsule of the *Streptococcus mucosa* which tends to decrease under artificial cultivation, as do also the capsules of pneumococci and streptococci.

Now, it seems to me—and this view has been supported by Walker, Deutsch, Welch, and Eisenberg, and is, in fact, but an axiom which would be recognized immediately by any trained

biologist—that all micro-organisms will adapt themselves so far as is permitted by their physiologic peculiarities to the stress of the environment, the exact direction which this adaptation will take being determined by the character of the environment, chemical and physical, and the physical, chemical and physiologic characteristics of the germ involved.

Thus we might have a germ increasing its toxin output in the face of neutralizing stimuli. Or we might have germs which are more especially opposed by lytic bodies, either increasing their receptor apparatus, as has been claimed by Pfeiffer in the case of virulent cholera organisms; or a more efficient protection can be conceived in the formation or increase in thickness and impermeability of surrounding envelopes, or extruded neutral substances, which are not soluble, but remain adherent to the germs. Eisenberg has developed this latest idea in his ectoplasm theory, and, indeed, it has long been recognized that capsule formation may be simply a response to unfavorable environment and a more or less efficient attempt at protection. It is certainly true that a number of our most highly adapted invasive germs, such as anthrax, pneumococcus, and the organisms of the hemorrhagic septicæmias, do develop capsules in the animal body; and the most virulent strains, it appears, have this formation most highly developed.

The fact also that many of the non-capsule-forming organisms appear larger in the fluid of fatally infected animals lends some support to such a view.

DEFENSIVE FACTORS OF THE ANIMAL ORGANISM.

Thus far, in considering the means of offence and defence at the command of the bacteria, we have largely left out of consideration the animal organisms against which these are directed in an adaptive way, or by the changes in whose functions, metabolism, tissues, cells, and fluids, we are largely made aware of their existence.

The internal defences of the animal body—and with these alone we are concerned—have largely been elucidated through morphologic investigation of cellular activities taking place in

the animal body or under controlled conditions in the test-tube, and by visible reactions taking place in test-tubes between the fluids of normal or immunized animals and the bacteria and their products, and, finally, by the more purely physiologic tests of the protecting power and mechanism of action of animal fluids or extracts when introduced into another animal of the same or different species, along with the bacteria or their products.

Such studies have, as is well known, afforded a vast amount of information. Through them the soluble secreted bacterial poisons have been demonstrated and have been found to stimulate the production of neutralizing bodies, the antitoxins; bacteria and their culture filtrates have been shown to call forth bodies which are present in the serum of animals treated with them, and which cause a precipitation of certain bacterial constituents of the filtrate—the precipitins; and injections of animals with bacteria or their products have been found to cause the production of bodies which are present in the serum and which have the power of agglutinating the bacteria when brought into contact with them—the agglutins; and other bodies are likewise produced which are capable under proper conditions of killing the bacteria—the bactericidal substances; or even of dissolving them as we have seen in some instances—the bacteriolytic substances. All of these bodies may be demonstrated in the serum of certain normal animals and may be shown to be increased during the immunization of these animals with bacteria or their products. The complementing body, however, which is necessary for the activation of the bactericidal and bacteriolytic bodies is not known to be increased during immunization, at least so far as its presence in the serum is concerned.

These facts we have learned from the study of the serum; on the other hand, the morphologic investigations instigated and carried on largely by Metchnikoff and his followers have taught us the great part which the formed elements of the blood and lymph play in the protection against and cure of germ diseases, and the importance of the polymorphonuclear

and large mononuclear leucocytes as phagocytes is now widely recognized.

Of these cells, the polymorphonuclear leucocytes take a very active part in the ingestion and destruction of bacteria, while the large mononuclear leucocytes and endothelial cells, especially those lining the blood-vessels and body cavities, although also able to ingest bacteria directly, are chiefly active in taking up cells of animal origin, principally those which necessarily, in the normal course of events, belong to the same animal and have probably become injured or have suffered death.

It is not, in this connection, to my mind, a far-fetched idea to suppose that phagocytic cells may depend naturally on other cells and bacteria as a part of their regular food supply. The polymorphonuclear leucocytes may thus depend to some extent on the ever-entering bacteria and their remains; for, as we know, bacteria are constantly entering along the regular channels of absorption; and it is just as obvious that numbers of blood- and tissue-cells are constantly dying out and must be disposed of, for such processes are always in evidence in the spleen, and the ingestion of polymorphonuclear leucocytes by the large mononuclears can be observed wherever leucocytes are collected in exudates, due either to infections, poisons, or supposedly benign irritants. The simple fact that these cells retain the basic physiologic activities and an ability to ingest and digest food in its crudest form, which ability was the heritage of their free-swimming ancestors, and that they have not suffered the total specialization and physiologic degeneracy of the fixed tissue cells, is sufficient evidence to my mind to warrant the conclusion that they are the most active factors in the protection of the specialized internal tissue-cells, which control the general metabolism and higher functions of the animal body. I would call attention here to the fact that the leucocytes, alone probably of all the true cells of the body, are entirely independent of the nerve control, are subject alone to the stimulation of their chemical and physical environment, and are thus susceptible of adaptation to various purposes which would be

fatal to the duties of cells controlled by the nerve mechanism for the special functions of the organism at large. Further than this the death of leucocytes does not matter, as would the death of specialized and nerve-controlled cells, for no special metabolic or functional derangement occurs from their destruction.

By citing this independence of the leucocytes I would not have it understood that they have not varied from primitive amœboid cells, for undoubtedly their life and proper functioning is largely determined by the special plasma in which they live, and it may be that their food, although at times crude compared with that of the other body cells, is nevertheless often prepared for them by processes going on in the plasma.

Questions relating to the independence and to the interrelation of the plasma and leucocytes in their action on invading micro-organisms and the action of plasma as compared with serum have been ground for scientific strife for many years, one side hotly contending for the activity of the plasma, the other for the activity of the phagocytes; the humoralist at first neglecting, if not absolutely forgetting, that a fluid can not be self-replenishing, while the supporters of phagocytosis largely overlooked the fact that plasma is not necessarily an inert menstruum such as salt solution.

While these differences have been to some extent adjusted by the theory and work of Ehrlich, an immediate point of contention is still the question of the similarity of action of plasma and serum. The humoral school contends that the alexin of Buchner—complement of later writers—is secreted into the plasma, while the Metchnikoff school claims that it is only given up from injured leucocytes in the body, and to the serum during coagulation. The Metchnikoff school admits, however, that the amboceptors necessary for bactericidal and bacteriolytic action are formed in excess in the phagocytes, and given off from these to the plasma, yet asserts that they are inactive for lack of the complement which is normally retained in the leucocytes, and that they simply prepare the bacteria for complete digestion in the leucocytes. The relation of the bacteriolytic

amboceptors to intracellular digestion is not settled, although it seems illogical for a digestive body to be produced in excess that has not arisen from cells by the stimulation of its use, and, as the leucocytes take up the bacteria, they are the most likely producers and users of this body.

In 1894 a further adjustment of differences took place, when certain phenomena observed by Denys and his pupil Leclef demonstrated that the act of phagocytosis when performed in serum, in some instances at least, was dependent on the presence of substances in the serum. Thus they were able to show that leucocytes removed from normal blood and placed with bacteria in immune serum engulfed the bacteria actively, while leucocytes from immunized animals mixed with bacteria in normal serum took up the organisms no more actively than the normal leucocytes. The bodies inciting the phagocytosis must obviously, then, they concluded, be in the serum. Whether these bodies acted on the leucocyte or on the bacteria was not then determined, but Denys concluded, in 1898, that the bacteria were directly affected. The fact that the action is exerted on the bacteria was recently determined positively by Wright for normal serum, and by Neufeld and Rimpau, independently of Wright, for immune serum. These bodies have been called opsonins by Wright, and bacteriotropins by Neufeld, and have been shown to attach themselves to the bacteria and thus prepare them for ingestion by the phagocytes. It has also been shown by various observers that the more virulent the germ, the less susceptible it is to phagocytosis and the more potent the antisera must be to permit of the ingestion by the cells.

If now, for clarity of conception, we summarize briefly the disease-producing agents possessed by the bacteria and the opposing substances of the serum and processes of the animal body, we find the true toxins, including probably leucocidins and hæmolysins, opposed by antitoxins which become free in the plasma; the bacterial bodies and probably the endotoxins opposed by leucocytes, and possibly directly in the plasma by lytic substances formed of amboceptor and complement, which either kill or dissolve the bacteria and free the endotoxins, but

do not neutralize them; and, third, we have probably certain secretions which oppose the opsonins, and thus prevent phagocytosis—antiopsonins—bodies which may possibly be the so-called aggressins of Bail, and which are present in exudates and, although not toxic in themselves, increase the infectiousness of the bacteria with which they are injected; and, finally opposed to bacteria and their broth filtrates we have the agglutinins and precipitins, the activities of which are manifest in serum, but whose relation to immunity is not altogether obvious, as they have not been shown satisfactorily to bring about agglutination or precipitation in the animal body.

While all of these different functions and chemical substances are possessed by animals as a class, it is becoming more and more obvious that these are not always present or active in the same degree, and that there are recognizable differences in the protective mechanism of different animal species—in species, in fact, not far removed from each other in the natural classification. An explanation of reactions to a given infection which applies in the case of one species is not, therefore, obviously applicable in the case of another species. This is true not only of the mechanism of protection as it takes place in the serum of different animals and in their plasma, but also of phagocytosis and phagocytic digestion and the factors which contribute to the perfection of these processes. The constant stumbling-block in the way of a correct interpretation of processes going on in the animal body is our inability, as we have seen, to argue from serum phenomena to phenomena occurring in the plasma. A failure to keep this in mind, although it is fully recognized, has undoubtedly led to many hasty conclusions, particularly connected with the theory of lytic immunity. Let me illustrate this with a well-known example: Fresh rabbit serum is actively germicidal for anthrax bacilli, dog serum is not; yet rabbits are extremely sensitive to a true anthrax infection, while dogs are very resistant. Experiment has shown that there are lytic amboceptors in the sera of both these animals, but that the dog's serum does not contain the complement necessary for their action on the bacilli; the complement presumably has remained

in the body cells, whereas in the case of the rabbit it has possibly been liberated from the leucocytes during clotting. The reason the dog is insusceptible is, then, not because of a more active plasma destruction of the invading anthrax germs, but because of a more perfect adjustment of the cellular mechanism to the infection, although if we simply followed the theory of the bactericidal action of serum and plasma as being coextensive, and the active protective mechanism, the rabbit should have been protected, while the dog should have succumbed. The difference probably resides in the possession of all requisites for the perfect performance of phagocytosis, and the complete digestion of the bacteria by the phagocytes of the dog, while in the rabbit either the mechanism of ingestion is incomplete or the cells fail to cope successfully with their contents after ingestion. This example has been selected because anthrax bacilli have been shown to contain less toxic intracellular bodies —endotoxins—than many other infectious germs, and the likelihood of the rabbit being poisoned by any primary plasma disintegration of the bacilli is not very great, so that if the plasma mechanism had corresponded to that of the serum the animal should have been saved. The validity of such an argument would not have been so apparent if we had substituted cholera vibrios for anthrax bacilli in rabbits, for the bodies liberated from cholera bacilli at their disintegration are very toxic.

Even if we were willing to admit on the evidence so far in our possession that the bactericidal and bacteriolytic bodies which are present in the sera of various animals are present and active against certain micro-organisms in the same manner in their plasma, we should, nevertheless, still have a number of micro-organisms which are singularly insusceptible to such action of the sera or plasma, even of animals highly immunized against them. The method of resistance against these would have to be explained by a different mechanism, and I do not wish to include here simple insusceptibility to the action of their poison, but an actual destruction of the bacteria and their poisonous products. Now, if this death and destruction is not accomplished in the plasma, then, unless we postulate a

direct excretion of the bacteria by the liver or kidneys, for instance, an occurrence which is really a pathologic phenomenon, not a process of cure, we must look largely to the activities of the leucocytes for its accomplishment.

The serum substances which further leucocytosis have, as we have seen, received much attention of late, and the bodies antagonistic to the bacteria which are supposed to be contained in the leucocytes have also been extensively investigated.

Experiments bearing on these questions make it appear extremely probable that bactericidal and digestive action depend on two processes; one of these is the bacteriolytic action of the serum and plasma, the other the bactericidal action of substances retained in the leucocytes. As an example of the type dependent solely on the bactericidal substances of the serum or plasma, the mechanism of the natural and artificial immunity of guinea-pigs to typhoid and cholera may be cited, since in these animals no one has as yet succeeded in demonstrating that substances derived from the leucocytes by extraction have any bactericidal action on the organisms of these two diseases. This does not mean, however, that the bactericidal action takes place naturally outside of the leucocytes, for the bacteria loaded with amboceptors are probably taken into the leucocyte and there digested. As examples of immunity depending on the bactericidal substances of the leucocytes, the natural and artificial immunity of dogs and cats to anthrax, and the immunity of guinea-pigs to certain strains of proteus, may be cited, for in these cases the leucocyte extract is germicidal, the serum is not.

Stated impartially, then, our knowledge of immune bodies and processes stands somewhat thus: Bodies which are bactericidal and bacteriolytic may be present in the plasma, and certainly in the serum, wherever this is formed in a pathologic process, which when supplied with complement, either normally present in the plasma or derived from injured leucocytes or other sources, may be active against micro-organisms, either killing them or actually breaking them up in some cases and liberating bodies which are then directly poisonous or become so

by further digestion. Besides these germicidal bodies there are other bodies which, while not directly harmful to the bacteria, render them powerless against the phagocyting power of the leucocytes. These bodies are probably present in the plasma, scarcely in the serum. They are the opsonins or bacteriotropins.

After phagocytosis has taken place, the germs may be killed and digested. Some of the bactericidal bodies of the phagocytes are bodies differing in character from the lytic bodies of the serum, and are either not given off to the serum or are not active in it; but there is no proof that the lytic amboceptors present in the serum are not normally derived from the leucocytes and active in intracellular digestion when activated by complement. This is supported by the supposition that guinea-pig leucocyte extracts are not germicidal for cholera and typhoid organisms. Nevertheless, intracellular digestion of these germs does go on: it is likely, therefore, that the amboceptors present in the plasma, whatever their source, attach themselves to the germs and aid in intracellular digestion.

None of the processes just mentioned leads to the formation of antitoxins which become free in the plasma or serum. Now, in view of these facts and suppositions, I believe that it is logical to conceive that nearly all pathogenic germs secrete bodies which are not readily soluble in culture fluids or in the fluids of the animal body; that these bodies are not readily, if at all, assimilable by non-phagocyting cells. These bodies may, however, be broken up by digestive bodies present in the serum, and from them may thus be liberated a poisonous body, which may then be assimilated by the higher cells of the body, and, when in sufficient quantity, cause death. The more rapid the process of liberation the more quickly death ensues. This plasma digestion is, then, according to my conception, a mechanism which is faulty when applied to bacteria. I conceive that the fault occurs somewhat as follows: Bacteria and their insoluble or non-assimilable products when taken into the phagocyte are subjected to two processes, a primary bactericidal and coagulating one, and then a more leisurely lytic or disintegrat-

ing action, during which poisonous products are probably liberated, but slowly enough to be taken care of by neutralizing bodies. Even if the leucocyte dies, it is usually promptly taken up by a mononuclear cell, and the poisons do not become free in the fluids. Now, in this process the only bodies which are produced in excess and at the same time are capable of escaping from the leucocytes are the lytic bodies; neither the toxin-neutralizing or toxin-destroying body nor the coagulating body are secreted or given off from the cell. Such lytic amboceptors, then, when present in the plasma and activated by complement, may become an active agent for harm by liberating poisonous substances from the bodies of germs which are susceptible to such action, or from the insoluble or non-assimilable products of these or more lysis-resisting members of the invasive organisms; and by the action of these poisons, phagocytosis may be hindered and the specialized cells poisoned. Since the neutralizing or coagulating bodies are not present in the plasma, the leucocytes are then poisoned from without, just as are the specialized cells, and the more active the plasma digestion the more deranged the true protective mechanism becomes.

I have gone thus somewhat lengthily, but nevertheless in an outline manner, into some of the problems of immunity, particularly those relating to the micro-organisms which are harmful to the animal body, not so much through their ability to secrete harmful soluble poisons, as through their insistently invasive character, or by the liberation of the toxic products resulting from the destruction of their secretions or of their own bodies. It is the diseases caused by these organisms on which I now wish to centre attention.

The organisms of these diseases undoubtedly belong to two or more classes, in one of which I would place the typical septicæmia producers—anthrax, pneumococcus, streptococcus, etc.—in the other the non-invasive organisms, typified by cholera and to some degree by typhoid. Between these two extremes there are all grades. The bacillus of tuberculosis, I think, stands by itself and offers a most special problem.

If I have made myself at all clear in setting forth some

of the data amassed in the study of these types of micro-organisms, and of the processes supposed to be involved in meeting infection and establishing cure and immunity from them, I think you will comprehend more readily some of the problems which daily face us in our struggle to arrive at a rational method of biologic treatment, and realize more fully, in the light of this knowledge, why disappointment has so persistently followed in the wake of serum therapy. For, in spite of the most persistent attempts to produce curative sera, the results have not been satisfactory and have not led, except in rare instances, to the practical use of such sera in the treatment of disease in man.

The sera, thus produced, have not, except in a very minor way, been antitoxic in the usually accepted sense, and depend, as we have seen, probably, for any protective value they may possess, on their germicidal and bacteriolytic power and on the opsonins they may carry, and thus facilitate phagocytosis. These sera are capable of protecting an animal from a many times lethal dose of an infecting organism, when mixed with it in surprisingly minute quantities; but that any consistent curative effects, other than merely local, have been definitely determined as due to their action, after an infection has once been established, may justly be questioned.

On the other hand, indeed, test and experiment have shown that animals and man suffering from a true infection may and often do themselves furnish sera capable of strong bactericidal and bacteriolytic action (when combined with normal sera containing complement), and yet, in spite of this, they succumb or may be subject to severe relapses.

In the light of these and other facts which have been cited, it has long seemed to me that one might well refrain from attempts to produce beneficial effects by injecting still further amounts of bacteriolytic or similar bodies, and seek further for an explanation of the exact methods and processes of the cure effected in those animals and man who do survive an infection.

Failure to solve these problems on lines hitherto followed

should not discourage us, however, while we know that animals and man do recover naturally from such infections and that the mechanism of the animal body suffices to protect the animal even against enormous doses of injected organisms, without serious histologic changes or marked systemic symptoms, if these organisms be given at proper intervals and in gradually increasing amounts. The conclusion that this power must reside in increased digestive and neutralizing or poison-destroying powers of the animal organisms cannot well be avoided, and these functions of the animal mechanism will probably be found to take place largely in some group of cells.

The animal body then, ideally protected in the time of bacterial invasion, may well be one in which some set of cells—phagocytes—are immediately ready and able to take up the bacterial invaders and destroy them, and within their own bodies to neutralize or destroy the action of any poisons secreted by such invaders or arising from their destruction by digestion, and this without serious harm to the ingesting cells; or—failing this full immunity from serious harm—it may be that these ingesting cells are, in their turn, taken up and, with their noxious contents, digested by other scavenging cells, with a minimum liberation of the substances which could injure the body cells dedicated to specialized functions. And I believe that the whole struggle of the infected organism may be summed up as a conflict between the leucocytes and the germs, and that it is an attempt to bring the invading germs within the leucocytes, and is a process with which the system at large often has little or nothing to do, except as an innocent and injured bystander, and that extracellular destruction of bacteria and toxicogenic bodies is an untoward event often leading to dire consequences, and depending on the chance occurrence of suitable digestive bodies in the serum which have been thrown off in excess from the cells, and may thus become a menace to the system at large by liberating poisonous bodies from comparatively harmless compounds.

Thus, in many instances, it seems to me, we are probably dealing with an immunity, a large part of the mechanism of

which is individually cellular, not only in the sense of phagocytosis and digestion, but in the neutralization or destruction of poisons which arise from the disintegration of the bacteria and their products—a mechanism in which the protecting cells *must* intervene and, unaided by antitoxic bodies in the plasma, neutralize within themselves the poisonous products of the invading micro-organisms.

It was this thought which suggested to me the idea of treating disease by aiding the leucocytes by furnishing them as directly as possible with the weapons which were being taken away from them in their fight with invading micro-organisms, and to protect them thus from destruction and give them an opportunity to recuperate and carry on successfully their struggle against the invading germs. These weapons, whatever might be their nature, I assumed might possibly be furnished by an extract of the active substances of the leucocytes themselves—substances not ordinarily given up to the plasma or serum—and I also assumed that extracts would be more efficacious than living leucocytes themselves, introduced into the infected animal, since, if diffusible, they would be distributed impartially to all parts of the body by the circulatory mechanism and, as quickly as absorption would permit, relieve the fatigued leucocytes and protect, by any toxin-neutralizing or other power they might possess, the cells of highly specialized functions.

This idea of immunity differs from one that simply assumes the cells as the source of all immune bodies in that it takes into consideration the presence and production in the leucocytes of agents which are not normally given up to the plasma, but which are constantly able to reproduce themselves and carry on the functions of coagulation, of digestion, or of neutralization simply for the benefit and protection of the individual cell, while not being secreted or excreted by the cells for the more general benefit of the cell community at large.

It seems to me, then, that when these sources of protection are overtaxed or fail to act efficiently on account of some inherent weakness or untoward circumstance of location, the most

reasonable course is, if possible, to support the chief army of attack, by introducing into the infected animal or man the *substances* composing the chief cells or all the cells of an exudate from normal or immunized animals in the most available and diffusible form, as little changed by manipulation as possible. The extracts of exudates from previously immunized animals might better serve this purpose than those from normal animals, since their cells probably have in their fight against the same organisms gained increased powers, as is evidenced by the ability of such immunized animals to dispose safely of immense numbers of organisms without serious harm or loss of weight. As this might, in part, however, be due to bodies in the fluids causing more rapid and complete phagocytosis, a further adjuvant, immune serum, might be found serviceable in some cases, especially if it were an anti-infectious or opsonin-containing serum.

In carrying out this idea in our tests, not only polynuclear leucocytes and bone marrow, but also extracts of mononuclear leucocytes, lymph-glands and spleen have been experimented with, not only because they might be found to have a toxin-neutralizing effect, but because observation has shown that mononuclear leucocytes are active in engulfing certain organisms, such, for instance, as the bacilli of tuberculosis and certain organisms giving rise to chronic infections, and also parasites of animal origin.

It is obvious that such a scheme of experimentation is a broad one, and that some of its phases have been attacked from various sides by other workers.

Most of the workers have been chiefly interested, apparently, in elucidating the direct germicidal action of certain leucocyte extracts on bacteria, and in bringing out differences between the serum alexins and the bactericidal bodies of the leucocytes —in other words, with the visible mechanism of the bactericidal activities of leucocytes as shown *in vitro* and in the peritoneal cavity.

Attempts to influence infection by the use of organ extracts have also been made, apparently with little success. The

thought, however, underlying most of this work differs from my own, as these experimenters have adopted such a course, arguing that certain organs may be the source of true antitoxins, or of the so-called immune bodies—amboceptors—and simply contain them in greater quantity than does the serum.

My work, on the other hand, as I have endeavored to make clear, is based on another conception, and has had as its immediate object the practical determination of the *curative* effects of such extracts.

THE TREATMENT OF INFECTIONS IN ANIMALS WITH LEUCOCYTE EXTRACT.

Passing, now, from these questions of theory, I will outline briefly the result of the treatment of infections in animals with leucocyte extracts. These infections or poisonings have usually been brought about by intravenous inoculations, and, as a rule, the treatments with extract have been given subcutaneously, although at times intraperitoneally.

The infections to which I wish to call your attention chiefly are: staphylococcus, streptococcus, pneumococcus, typhoid, dysentery, meningococcus and cholera, although we have also experimented with tuberculosis and with infections due to micro-organisms of animal origin—the spirochætes and trypanosomes—and with animals suffering from inoculable tumors.

It is evident that the extracts used must vary in strength, as there is no means by which they can be exactly standardized, principally on account of the red blood-cells which are often present in the exudates.

I would call attention here to the fact that neutrophilic granules of the rabbit cells apparently go into solution in water, the cytoplasm undergoing marked disruption, while the nuclei of the cells swell up and resemble the larger, paler nuclei of mononuclear cells, but by no means go into complete solution. It would not surprise me, therefore, if the constituents of the cytoplasm were active. What part the nuclei play in the outcome of experiments is a question I am at present unable to answer, although I am not unmindful of the beneficial action

claimed by various writers for the so-called nucleates (nucleic acids), especially in warding off peritoneal infections. They, however, refer this action to an increased leucocytosis. The sources of these nucleic acids are, however, so varied, being either from yeast or animal cells, that one might well, theoretically at least, look askance at the introduction of some of these bodies during the course of a disease already taxing the powers of the system.

Staphylococcus Infections in Animals.—If we analyze our series of staphylococcus infections,[1] we find that animals receiving subcutaneous injections of rapidly fatal doses of *Staphylococcus pyogenes aureus* can generally be saved by treatment with the extract of normal leucocytes of rabbits even in small doses, especially when these are given intraperitoneally. When intravenous injections are practised, the results are different, but treated animals usually survive the controls many days and present modified histologic pictures.

Streptococcus Infections in Animals.—In experiments with streptococcus infections I have worked only with those brought about by intravenous injections. In these experiments we find a marked lengthening of life and even a survival of the treated animals, and better results in the animals treated early than in those treated late. All of the animals which lived long enough developed articular or periarticular lesions, which tend to distinguish these infections from pneumococcus infections, which, as we shall see, seldom or never give rise in rabbits to such localizations.

Pneumococcus Infections in Animals.—If, in my series of experiments on pneumococcus infections, we consider the animals treated with the extract of leucocytes of normal rabbits, we find that in such animals an infection, surely fatal in untreated rabbits, becomes significantly modified in treated animals, even if this treatment be delayed many hours. Thus, out of eight control animals used in four experiments in which

[1] Hiss: Jour. Med. Research, 1908, xix, 323. See this article for details of these and the following experiments on animals.

the intravenous infecting dose was the same, all died, averaging only forty-five hours of life after being infected. Of the animals treated—some as late as twenty-four hours after infection—nine out of twelve survived the infection, three died with an average life of sixty hours after infection, two of them not having received treatment until the expiration of twenty-four hours.

These are not selected examples, but are records of events as they developed in our regular research tests, and have been fully confirmed by experiments undertaken in elucidation of other points, and are unmistakably indicative of the powerful beneficial action of such extracts on pneumococcus septicæmia in rabbits.

On the other hand, living leucocytes, introduced subcutaneously, or even peritoneally, have little or no effect on systemic infections.

Typhoid Infections in Animals.—We find that typhoid infections, if indeed we may really call them such, in rabbits are essentially different from infections caused by such organisms as staphylococci, streptococci, and pneumococci. The animals seem rather to suffer an acute intoxication, from which they either die within a very limited time when organisms may be recovered from them, or recover completely, or go into a state of cachexia, but without organisms in the blood or organs. In my experiments the animals, as is usual after toxic doses of typhoid bacilli, showed signs of poisoning, remaining quiet and refusing all food for some hours. The animals receiving protection with leucocyte extract, shortly after this treatment, usually seemed worse than the control, and to the inexperienced would appear the most likely to die. This might possibly be due to a more rapid liberation of toxic substances by enhanced bacteriolytic processes, either brought about by a fuller complementing of immune bodies by the extract or to special digestive bodies of the leucocytic extract, or it might be due to a somnolent state following a relief from active poisoning. That the poisoning in reality was fundamentally less severe than in the more normal-appearing control is, however, shown by the rapid return

of the treated animals to normal condition and weight, the weights following a perfectly logical order—untreated animal, animal treated late, animal treated early, as is illustrated by the following examples:

	Untreated Animal.	Treated Within 5 hours.	Treated Within 1 hour.
April 8	1,125 Gm.	1,108 Gm.	1,104 Gm.
April 11	1,035 Gm.	1,114 Gm.	1,135 Gm.
April 12	1,030 Gm.	1,160 Gm.	1,200 Gm.
April 13	1,010 Gm.	1,125 Gm.	1,190 Gm.
April 15	1,085 Gm.	1,148 Gm.	1,225 Gm.
	(Injection.)	(Injection.)	(Injection.)
April 17	1,020 Gm.	1,130 Gm.	1,185 Gm.
April 18	1,000 Gm.	1,188 Gm.	1,250 Gm.
April 19	968 Gm.	1,160 Gm.	1,228 Gm.
	(Injection.)	(Injection.)	(Injection.)
April 22	935 Gm.	1,100 Gm.	1,135 Gm.
April 24	900 Gm.	1,100 Gm.	1,170 Gm.
April 26	865 Gm.	1,085 Gm.	1,210 Gm.
April 29	Dead.	1,070 Gm.	1,265 Gm.
May 1	——	1,140 Gm.	1,305 Gm.

In this experiment no organisms were alive in the control, so that the animal died from poisoning and interference probably with metabolism and excretion due to cellular changes.

In another experiment the dose of typhoid bacilli was so toxic that the temperature fell immediately instead of rising. The effect of the extract in the early treated animal was not a further lowering of the temperature, but an arrest and more abrupt rise than in the control and in the animal treated after five hours. Furthermore, both the control and the later treated animal had bad diarrhœa within two hours, but the animal treated in one hour did not have diarrhœa.

Such experiments with leucocyte extracts on typhoid infections in rabbits are sufficient for illustration, and the conclusion from them seems unavoidable that leucocyte extracts have a markedly beneficial modifying action on the course of typhoid infections or poisonings in rabbits.

The same holds true of infections in guinea-pigs treated with rabbit leucocyte extracts, but, apart from noting the fact here that subcutaneous injections, used curatively, are active in

guinea-pigs, it does not seem of import to detail such experiments at this time.

Meningococcus Infection in Animals.—In meningococcus experiments rabbits suffer a marked intoxication, if not, indeed, in some instances, a true infection, when given sufficient quantities of almost any race of meningococcus intravenously. During the epidemic of cerebrospinal meningitis occurring in New York several years ago I proved this to my satisfaction many times, so that for the purpose of the following experiments I had no hesitation in selecting rabbits as the test animals.

If we analyze briefly the results of our experiments on rabbits infected with meningococcus we find the following:

In every experiment the controls died.

The total number of control animals used in the experiments, in which the treated animals received normal lucocyte or immune leucocyte extract, was nine. In one of the experiments one of the animals was greatly over weight, and should not have been used. The animal died, however, in thirteen days. Leaving this animal out of account, we have eight controls averaging 1254 grammes, with an average life of twenty hours after infection.

Of the treated animals there were thirteen. Nine of these recovered and four died, over 70 per cent. of recoveries.

The average weight of the nine animals which recovered was 1200 grammes, and of the four which died 1062 grammes, 200 grammes lighter than the controls, with an average life after infection of 5.7 days.

The majority of the animals did not receive treatment until the expiration of five hours after inoculation, and a number of them not until twenty-one to twenty-four hours, some of the controls having at times died before these animals were treated with leucocyte extract. Severer tests could hardly be devised, and when the results of such tests are compared with those obtained with the use of serum they point strongly to the value of leucocyte extracts in the treatment of this infection.

Cholera and Dysentery Infections in Animals.—Experiments with cholera infections of guinea-pigs and rabbits, and with

infections due to either type of the dysentery bacilli in rabbits, which always proved fatal in the control animals, have shown that these infections yield to treatment with leucocyte extract, in much the same manner as do the infections to which I have already called attention.

SUMMARY AND CONCLUSIONS FROM ANIMAL EXPERIMENTS.

The character of the exact action of the extracted substance in these various infections is, of course, at present largely a matter of conjecture. The fact that treated animals, in some instances, appear more intoxicated than the untreated, may indicate an enhanced lytic action and liberation of toxic substances, thus suggesting the presence in the extracts of complementing bodies, or of digestive bodies peculiar to the leucocytes. These bodies may, of course, be present and play an active part, but the strongest impression given to one carefully following the experiments, and noting the immediate effect on temperature and the conservation and quick return to normal weight of the treated animals, is that the principal substance at work is one active in neutralizing poisons, destroying or preventing their formation, and thus able to relieve the animal economy and give the phagocytes an opportunity to carry on their work of ingesting the micro-organisms and their non-soluble products, thus permanently rendering them harmless.

The points which to my mind at present speak most strongly for the explanation of the beneficial action of leucocyte extracts, as either poison-neutralizing, poison-destroying, or poison-prohibiting, rather than primarily bactericidal or bacteriolytic, or even one of immediate phagocytosis, are first, the effect on temperature; for, when the temperature is high it tends to be lowered, and when it is falling below normal from the intensity of the poisoning it tends to rise, on treatment; which is a sign, of course, under this condition, of returning strength on the part of an animal; and, secondly, the effect in preventing diarrhœa, which is a symptom of poisoning in such intoxications. Animals treated in time rarely show diarrhœal symptoms. Now, if the action of the extract were a lytic or a poison-liberating

one, we might possibly have a fall in temperature when it is high, following the introduction of extract, caused by a further poisoning, but we should not have a rise in temperature in an already fatally intoxicated animal; in the same way, if poison were liberated, animals receiving treatment should envince symptoms of diarrhœa before the untreated ones. And, further, the experiments performed by Zinsser and myself *in vitro* and in the peritoneal cavities of guinea-pigs strongly favor a toxin-neutralizing or coagulating action, for we were able to show, in a fairly satisfactory manner, that bacterial extracts were precipitated by leucocyte extracts, and we were unable to determine any marked extracellular intraperitoneal destruction even of such sensitive organisms as cholera vibrios, or, in fact, any immediate increase in phagocytosis; and yet the animals were better off than the controls. That the action is one affecting the poisons immediately, no matter what the effect on the bacterial cells may be, seems an unavoidable conclusion, since falls of temperature of several degrees may occur within a half hour to an hour after subcutaneous administration of leucocyte extract. This fact also practically rules out an explanation of the extract as being simply a stimulator of general leucocytosis from the leucocyte-forming bases of the animal; for, even if such a leucocytosis could occur in so short a time, we know from experiment that freshly obtained living leucocytes, when introduced into an infected animal, even intraperitoneally, are practically without effect on systemic infections. The lives of the animals are not lengthened and these intact leucocytes seem to have no influence on the temperatures.

Intravenous injections of living leucocytes have not been tried, since the results of such a procedure are of purely academic interest, being entirely outside the realm of possibility in the treatment of human infections. The use of living leucocytes in the treatment of local infections, such as those of the pleura or peritoneum, or even subdural infections, is, of course, possible, but of limited application, both theoretically and practically, and their beneficial action would probably be due to the

simple recruiting of the phagocyte army, or to extracts which, unsuspectedly, accompanied the supposedly intact leucocytes introduced.

THE TREATMENT OF INFECTIONS IN MAN WITH LEUCOCYTE EXTRACT.

While animal experiments are necessary, and often extremely instructive, one can, nevertheless, not argue directly from these to occurrences in man. An injection disease is not an infectious disease, and we are dealing usually with conditions in man which are at least not entirely analogous to artificial infections in animals. Artificial infections are usually accomplished by an abrupt introduction of a large quantity of infecting germs and their products; the animal powers of resistance are often immediately and severely taxed; the incubation period thus artificially shortened; and the germs themselves are not subjected to such a searching elimination, being present in large numbers, as is usually the case with the few organisms gaining a foothold by the natural channels of infection. This difference is most marked in septicæmias, in which, in animal experiments, the organisms have been introduced directly into the circulation in quantities sufficient to bring about a very rapid poisoning and overwhelming of the animal, with probably only a very partial adaptation of the bacteria to the animal agents of resistance. On the other hand, the septicæmic invasion in man most often follows the adaptation of the germs in some more favorable *nidus*, and probably has to do with an evolution in the bacterial resistance to the protective powers, rather than a decrease in protective strength on the part of man. Indeed, both of these processes may increase hand in hand, and we may have septicæmias extending over weeks, months, and even years. We may have, in fact, an "armed peace" and the prepared bacterial army is not to be routed by the application of means which under other circumstances might prove efficacious, for we have seen how the bacteria may possibly become resistant to the protective agents of the animal body, and may

continue to survive attacks which might well prove fatal to less well-adapted members of their species.

Theoretically, then, I think we are safe in assuming that the infections in man which most closely simulate artificial infection in animals are fresh local infections, and infections of any character, in their earliest stages, before the bacteria have been adapted to carry on their fight with the powers of the infected body. Septicæmias in man which simulate most closely those of animals are of a fulminating type, due either to a markedly virulent (already highly adapted) organism or to an unusually low state of resistance on the part of the patient.

The point which I wish, if possible, to make clear is that it seems to me that the outcome of our attempts to treat infectious diseases is, if we have the real means in our hands, probably more dependent on the degree of adaptation of the germs than the actual powers of resistance of the patient. These latter, of course, determine largely the picture of the disease, but give little information as to the power of the invader. This has been forced on me by the fact that, although we are able to cure positively acute septicæmias in animals by the injection of leucocyte extracts, the more subacute septicæmias of man do not yield readily to our present mode of treatment, whereas even extremely severe acute and chronic localized diseases, due to the same organisms, respond to such treatment. This is true of pneumococcus, streptococcus and staphylococcus infections. I have given the matter much thought and it has seemed to me that our hope here lies possibly in a combination of heterogenous immune sera with the leucocyte extract, since it is probable that these may be able to attack different receptors, and thus destroy the organisms or prepare them for phagocytosis, whereas man's own plasma has become powerless against them. This outlook seems encouraging when we remember that organisms rendered more and more virulent for one species of animal do not always increase in virulence for another species, and may even, as is well known, become distinctly less infectious for other species.

We have evidence of this from studies of the virulence of streptococci, which, no matter what their source, even from the

most rapidly spreading and fatal infections in man, may be of no particular virulence for rabbits; and, on the other hand, when rendered exquisitely virulent for rabbits, may prove practically benign when introduced in immense quantities into dogs. This is an illustration familiar to all bacteriologists and requires no more experimental evidence. We cannot, however, be certain that these differences reside entirely in the plasma immunity of the animals, so that we should probably combine the leucocyte extract of the immune animal with its own serum.

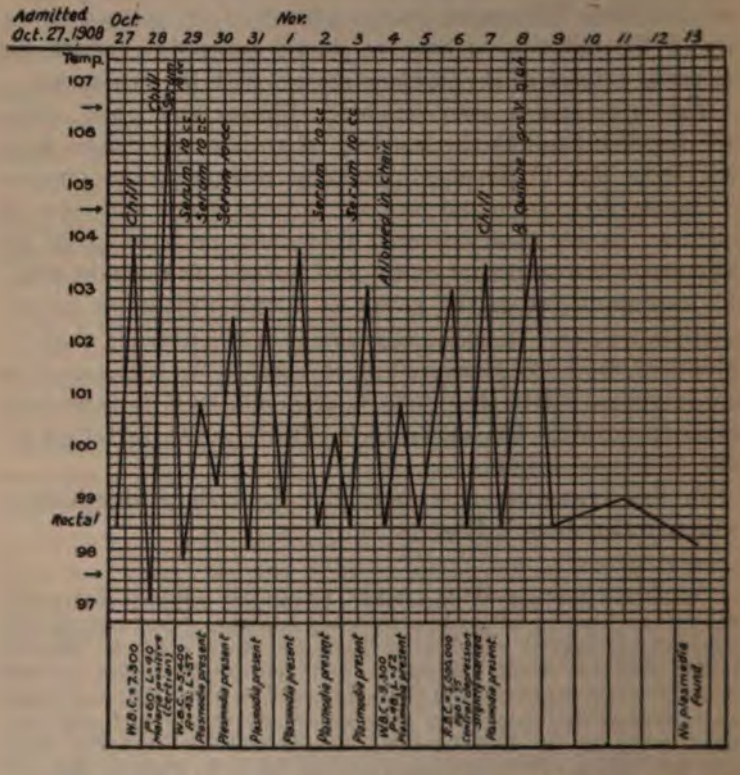
The infections in man which Dr. Zinsser and I and others have treated, through the courtesy of various physicians, have been those due to the meningococcus, pneumococcus, streptococcus, staphylococcus, gonococcus, typhoid, colon and tubercle bacillus. Beside these bacterial infections, we have tested the influence of the treatment on cases of malaria, as the most available representative of the group of known pathogenic animal micro-organisms. In such infections it is not unlikely that the extract of the large mononuclear cells plays the major part in any beneficial effect that may be observed, as it is these cells which experiment has shown to be chiefly active in taking up cells of animal origin.

In this connection I wish to cite the history of a woman patient in St. Luke's Hospital.

Case I.—*History.*—Two months before her present illness this patient had been in St. Luke's Hospital suffering from malaria and had been discharged cured after treatment with quinine. She remained well for about one month, but four weeks before present admission again began to have chills, followed by high fever and sweating. These at first came every other day at about midnight, and were accompanied by the classical symptoms of malaise, headache, backache and nausea. This continued getting worse in spite of the fact that she was taking ten grains of quinine every day, and had been doing so for about six months. About three days before admission to the hospital chills began to come every day, and were now occurring during the late afternoon. On admission to the hospital, on October 27, 1908, a chill occurred between four and five in the afternoon, her temperature reaching 104. The chill lasted thirty minutes. During the night the temperature began to fall again, and by noon on the following day had reached 97° F. It did not remain low for more than an hour, and between 2 and 4 o'clock another chill occurred, the

temperature rising abruptly to 106.2° F. A blood examination made at this time showed 7300 leucocytes, 60 per cent. polynuclears, and numerous tertian parasites present. Immediately after this second chill it was decided to attempt treatment with leucocyte extract, no quinine having been administered since the admission of the patient to the hospital.

CHART OF CASE I.



Treatment with Leucocyte Extract.—Ten c.c. of extract were injected subcutaneously, at about 9 P.M., about five hours after the highest temperature reached during the chill. The temperature during the night fell as usual, reaching 98° F. the following morning, when another 10 c.c. of extract were injected. A third dose was given at noon. On this day no chill occurred, the highest temperature reached being 100.8 about 6 P.M. No more leucocyte extract was given that day, but on the following day 10 c.c. were given about 9 A.M. The temperature on this day reached 102.2, but no chill occurred. Con-

omitant with the omission of the chills there was a very marked improvement in the general condition of the patient. Almost immediately after the first injection a reduction of the nausea, the malaise, and restlessness occurred, with complete alleviation of the headache, which had been extremely troublesome. Numerous blood examinations during this time showed, however, that there was absolutely no diminution of, or change in the development of, the plasmodia. During October 31 and November 1 no extract was given. No chills occurred, but each day the afternoon temperature exceeded that of the preceding day, on November 1 reaching 104° F. at 8 P.M. On November 2, at about noon, 10 c.c. of leucocyte extract were given, and on this day the temperature did not exceed 100.4. On November 3, 10 c.c. were given shortly after noon, and the temperature did not exceed 103, the patient feeling extremely well. On November 4, the following day, no further leucocyte extract was given, nevertheless, the temperature did not exceed 100.8, and the patient felt so well that she was allowed up in a chair. No further injections of extract were made, and on November 5 and 6 the afternoon temperature became gradually higher, reaching 103 at 6 P.M., and on November 7 a sharp afternoon rise to 104.2 was accompanied by a chill. This chill on November 7 was the first true malarial chill which had occurred since the beginning of treatment on October 28. During all this time the patient had been feeling extremely well and all the symptoms, which were analogous to those of toxæmia in malaria, had been alleviated. On the other hand, absolutely no diminution or change in the plasmodia was noted. On November 8, 5 grains of quinine were given every four hours, which, after a few days, resulted in complete cure and disappearance of the plasmodia from the circulating blood.

Although isolated, this case showed such a striking reaction to the first administration of leucocyte extract and such an unquestionable return of symptoms upon omission of the leucocyte extract that, while, of course, a single case warrants no conclusions, the evidence certainly favors the opinion that the extract had a distinct neutralizing value for the poisons elaborated by the plasmodia. It is interesting, moreover, to note that the symptoms of the disease were entirely alleviated without there being any visible effect on the plasmodia, a fact which seems to indicate that certain phenomena appearing during this disease may be interpreted as the results of toxæmia.

That malaria can be thus influenced would seem to be a fact of more than passing interest, and it is mentioned here as it holds out the possibility of the favorable outcome of the appli-

cation of such measures to the treatment of the spirochætal and trypanosomal diseases and other infections of protozoan origin in man.

There is not time, nor is this the place, to go into the histories of the individual cases, except by way of illustration of the various bacterial diseases which we have treated. In connection with Dr. Zinsser I have lately published the results obtained in the treatment of a number of meningitis patients, and in a small number of patients suffering from lobar pneumonia and pneumococcus infections, and to these articles I must refer anyone interested for details.[2]

Meningococcus Cases.—It may be of interest to state, however, that fifteen of the meningitis patients treated by us were under fifteen years of age; of these, three died, leaving 80 per cent. of recoveries with no sequelæ. Of the patients over fifteen years, there were seven, five of whom died. This result may have been due to the fact that several of these adult patients were *in extremis* when admitted to the hospital. In spite of this, under treatment some of the patients showed a marked improvement and did not die before twenty-seven, seven, thirty-eight, eleven, and twenty-five days after treatment was begun. Of the children who were treated, but who died, one survived seventy-nine days, one sixty-two days, and one, a baby of seven months, twelve days, after treatment was instituted.

It seems also of interest to note the fact that in seven cases in which treatment was begun *subsequent* to the seventh day of the disease there was 100 per cent. of recoveries without sequelæ. Treatment was not begun in these cases until the elapse of seventeen, twenty, forty-seven, forty-one, fifty-three, thirty-nine, and eighty-two days after the first symptoms developed, and a reference to the histories would show that these patients were, as a rule, in grave condition.

Almost without exception there was an improvement in those symptoms which, in this disease, depend largely on the central nervous system. Vomiting, delirium, stupor and hyperæsthesia

[2] Jour. Med. Research, 1908, xiv.

were usually diminished or entirely allayed after one or two administrations of quantities ranging from 5 to 20 c.c.

Marked reduction in the temperature following injections was noticeable in many of the cases. In some of these cases, however, the diminution of the fever was a temporary phenomenon, limited to the twenty-four or forty-eight hours immediately following the injection, a fact which also argues strongly for the idea of poison neutralization.

The actual percentage of recoveries among these patients was about 64 per cent. If 70 to 75 per cent. be accepted as the usual death rate, and the basis for determining the value of the serum treatment of meningitis, then the fact that 64 per cent. of our patients recovered would seem to indicate some curative value in the extract. And if this is true, it also indicates that if the antimeningitis serums are really toxin-neutralizing, or contain the same antibodies as the leucocyte extract, then these sera should be efficacious when administered subcutaneously, which is not the fact. I am inclined to believe that a combination of these treatments, immune serum given subdurally and leucocyte extract subcutaneously, might lead to more favorable results than the use of either separately.

Pneumococcus Infections.—In considering the effect of leucocyte extract on the course of uncomplicated lobar pneumonia it is impossible, in view of the small number of cases, to arrive at a definite opinion. Not more than twelve or fifteen patients have been systematically treated, and of these one has died. This might be true of any series of the same number. However, the uniformity with which temperature changes and the relief of toxic symptoms follow the administration of moderate amounts of extract in the lighter cases, and often in the severe cases after the administration of larger or repeated doses, points to a toxin-controlling and beneficial action of the extract. One case which has lately come under the observation of Dr. Patterson may serve as an illustration.

Case II.—Reported by Dr. Patterson. Mrs. G., 78 years old, had emphysema and chronic bronchitis with asthma; developed acute bronchitis.

January 4: Temperature 101.3. Diagnosis of probable pneumonia.

January 5: Very sick; evidences of toxæmia (character of pulse, cyanosis of finger-nails and lips, prostration) most marked. Looked as though patient would surely die. Seen by Dr. S. W. Lambert: Pneumonia; patient will probably die.

January 6: Patient about as yesterday, but condition not quite so toxic. At 3 P.M. patient cyanosed, appeared very sick, but not so much so as yesterday. Leucocytes, 10,900; polynuclears, 78 per cent. Twenty c.c. of leucocyte extract injected.

January 7: Distinct improvement in toxic symptoms; patient had developed appetite and asked for egg and toast. At 5.10 P.M., P. ex. Over left upper lobe in front, marked dulness; feeble breathing; but no change in quality of the voice and breathing. "Since first injection there is marked improvement in the patient's appearance and general condition, and a marked improvement in the character of the pulse." Leucocytes, 9200; polynuclears, 86 per cent. Twenty c.c. of leucocyte extract injected.

January 8: "Patient decidedly better." Dulness over left upper lobe less marked. Pulse of a better quality. At 3 P.M. 20 c.c. of leucocyte extract injected. Leucocytes, 9200; polynuclears, 90 per cent.

January 9: Patient's condition highly satisfactory. Twenty c.c. of leucocyte extract given. Course uneventful thereafter.

As an illustration of the effect on a patient in the very early stage, and possibly suffering from a less severe infection, the following is interesting:

CASE III.—*Patient.*—Mohan, male, adult, admitted to St. Luke's Hospital, service of Dr. T. C. Janeway. Family and previous history irrelevant.

Present Illness.—Four hours before admission, while at work, the patient had a shaking chill. His teeth chattered, his lips were blue, and he felt feverish. At the same time he began to suffer from pain in the lower anterior part of the right chest which was markedly aggravated by deep breathing.

May 10, 1907: Admitted to St. Luke's Hospital in late afternoon. Temperature, 102; pulse, 100; respiration, 26. Over the lower lobe of the right lung there were typical signs of consolidation. The sputum was blood-streaked. The leucocytes were 26,000; polynuclears, 89 per cent.

May 11: The condition of the patient was unchanged; temperature rose, reaching 104 at noon. Leucocyte extract, 6 c.c., subcutaneously injected. Following this injection there was a sharp and steady drop in the temperature, reaching 98.8 on the morning of the following day.

May 12: During the morning of this day temperature steadily rose, reaching 103° F. at noon. Leucocyte extract, 10 c.c., injected in early afternoon. Temperature again dropped, reaching 98 at midnight.

May 13: The general condition of the patient was very much improved, breathing was easier, pulse and respiration had become normal.

After a rapid convalescence patient discharged cured.

First injection was given on second day of disease. Total quantity given, 16 c.c.

This case is interesting primarily because of the apparently sharp and rapid response of the temperature to the injections of the leucocyte extract. The occurrence of the crisis on the third day of the disease is a phenomenon sufficiently rare to encourage the opinion that it may have been at least in part due to the injection.

Such occurrences as these, backed by the conclusive evidence of the animal experiments, would certainly seem to warrant an extended and careful study of the influence of this agent on pneumococcus infections in man and make us hopeful that many an otherwise fatal illness may become comparatively benign under its influence. This hope is strengthened by evidence gathered from the study of infections due to the streptococcus, so closely related to the pneumococcus in many ways.

Streptococcus Infections; Erysipelas.—Dr. Adrian Lambert in his wards at Bellevue Hospital, during last July and this January, has treated about fifty cases of erysipelas by this method. Erysipelas is the ideal infection to study, for the chief lesion is in plain view, and, besides, there is a marked temperature reaction to the infection. In adults the disease is not very fatal, but in children under one year of age the mortality is, I understand, very high. While the history of these cases will shortly be published in detail, Dr. Lambert has kindly drawn his conclusions for me. They are as follows:

Leucocyte extract will abort infections which are treated within the first forty-eight hours.

It will ameliorate the course of older infections and may abruptly terminate them; the longer the infection has existed, the less likely is the latter to take place, but it tends to shorten the course of the disease.

The toxic symptoms, delirium, headache, nausea and vomiting, are modified and relieved; local pain is lessened.

The rash does not disappear immediately, but is apt to be localized.

The spreading, intractable lesions of the back and body are apparently affected as readily as those occurring on the face and head.

Pus formation is aborted, and sequelæ are rare, if they occur at all.

About 50 per cent. of babies under one year of age have recovered from the erysipelas.

The effect of leucocyte extract on erysipelas strongly suggests its use in the streptococcus infections complicating the exanthemata, and, of course, in streptococcus infections in general. Many of the dangerous sequelæ of these diseases might be avoided by its use.

Staphylococcus Infections.—Local acute and chronic staphylococcus infections respond almost immediately to treatment with the extract, and furunculosis of intractable type is halted and apparently cured. Dr. Zinsser has treated eleven patients who had staphylococcus infection. In these, after treatment was begun, surgical interference was always unnecessary. The following history illustrates the response of chronic furunculosis to the treatment:

Case IV.—G. P., assistant carpenter, St. Luke's Hospital, 22 years old, Swede, athlete, general health excellent, for three years had been troubled with persistent pimples on the face. For the four months preceding examination he had had boils on the neck and behind the ears, a new one developing every other day. For the last few weeks boils had appeared on both legs and in the left axilla. For the last two weeks the patient had never gone two days without the development of a new furuncle. These had been growing steadily more severe, and during the last week he had developed a very painful furuncle with extensive induration within the external meatus of the right ear. On examination he was found to have at least fifteen to twenty boils on the back of the neck and behind the ears. *Staphylococcus pyogenes aureus* was cultivated from the boils.

October 7: Ten c.c. of leucocyte extract injected.

October 8: No new boils appearing and old ones improved.

October 10: Ten c.c. leucocyte extract injected. No new boils appear.

October 11: Ten c.c. given prophylactically.

No new development of furuncles until November 28, when a

small pimple appeared on the back of the neck, which was quite angry and painful, and 10 c.c. of leucocyte extract was injected.

December 1: No new boils had developed. The patient appeared entirely well, and has so remained till the present time.

As an illustration of the effect of leucocyte extract on an acute and severe infection, not well adapted to surgical interference, the following case may be cited:

CASE V.—Three weeks before present illness the patient, a woman, developed an acute dacryocystitis. There were several attacks of this condition following the introduction of a probe. There appeared to be a stricture in the nasal duct. *Staphylococcus pyogenes aureus* was at this time isolated from the inflamed area in the lachrymal duct and from the nose. Following an attempt to correct the stricture on January 12, 1909, infection from the nose travelled up through the nasal duct into the orbit. The patient's temperature rose to 103.4° F. and a rapidly spreading acute inflammation developed in the orbit, from the pus of which *Staphylococcus pyogenes aureus* was recovered in pure culture. There was marked exophthalmos, complete immobility and protusion of the eyeball. It was the opinion of the attending physician that the tissues behind the eye were œdematous and that the orbit probably contained pus.

January 13: Ten c.c. of leucocyte extract injected. Temperature dropped to 100.2 on the following day, but rose again to 101.8 in afternoon. On January 14, two injections of 10 c.c. of leucocyte extract.

January 15: Temperature dropped to 99.8. Marked improvement in general condition and in local lesion. During the night of January 15, temperature again rose to 100.8, but dropped on January 16 to 99. Ten c.c. of leucocyte extract injected. Temperature did not go above 100.2, on following morning being 99.4. General improvement progressed, and there was marked clearing up of local lesions. Rapid and uneventful convalescence.

Time will not permit me to call attention to work in other infections. Indeed, in the case of most of these the data are too scanty to warrant the expression of any definite opinions.

In conclusion, then, let me say that no one realizes more fully than I the vast amount of work, experimental and clinical which must be done before the value of the work presented to you can be fully determined and the crude weapons, new to our hands, be better fashioned and more skilfully handled.

HEREDITY IN MAN*

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WE hear to-day everywhere of conservation. Our rapidly diminishing supply of coal and ores must be husbanded; the soil which is to supply the crops for a hundred millions of Americans must be maintained in its fertility; and our forests must be protected from ruthless and selfish destroyers for our immediate needs, and new forests started for posterity. Those who are interested in our domesticated animals are active in preserving such races as possess particularly valuable qualities. The national government has even gone into the business of raising Morgan horses, to avert the threatened disaster of their total extinction. But there is one national asset to which too little attention is being paid, whose potentialities are being improperly developed, whose transmission to posterity is being threatened—the best of human protoplasm! In this city what special abilities in singing, in instrumental music, in art, in wit, in elocution! What individual capacities in remembering, in calculating, in administering, in constructing and inventing! What examples of skill, of patience, of pertinacity, of profundity, of grasp of details! If a census were taken of this audience, what a diversity of characters, what valuable assets of abilities, would be revealed. It is by no accident that they are here—they constitute our chief heritage from the past; they are the most precious of our national resources.

To make the best use of these resources, to transmit them in greatest proportion to posterity, it is esential that the method of their hereditary transmission should be perfectly understood. But the laws according to which particular characters,

* Lecture delivered March 6, 1909.

whether favorable for human progress or unfavorable, are transmitted, are as yet little known in detail. If an irresponsible autocrat, even the most learned man in the world in the lore of human heredity, were given the power of arranging future marriages, he would make a bad botch of it merely through ignorance. If we had precise knowledge of the inheritance of characters, if we knew the outcome of a particular mating with even the same assurance that we do that of the treatment for rabies, or diphtheria, or operable appendicitis, then we could rely upon a fair proportion of educated, reasonable people making a selection of their consorts with a fair regard for the probable nature of their offspring. The reason why there is no science of eugenics is, first of all, because we lack data as to the inheritance of particular characters in particular combinations. The reason why we lack data is not because they are not to be had; not, I conceive, because those in a position to do so are not willing to gather them; but because, until recently, we have not known how to use them after they are gathered. In rare cases have we been able to say that given parents with such and such characters, they will have such and such offspring; or that to get such and such a combination of characters in the child, the parents must be thus and so.

But we are in a new era now. At last, as a result of extensive experimental breeding, we know how to analyze hereditary data so as to draw definite conclusions from them. But we find the data which have heretofore been put on record to be far from satisfactory; both because of their meagreness and because of the inadequate way in which much that we have has been prepared.

You as medical men are peculiarly favorably situated both by education and by relation to the families of the community, to contribute data which can soon be worked up into definite conclusions. I think I may say as a result of some inquiry that there will be no difficulty in securing funds and the assistance of sufficient persons to collate and reduce any data that the physicians of this city may collect in the course of their routine work. I have reason for thinking that if they once

realize the certainty that important results can be gained from data that they can easily collect the physicians of this city will enter enthusiastically into the business of collecting such data. I am therefore very glad of this opportunity of giving to you some account of the more recent results in the study of heredity, especially in their application to man.

The first fundamental conception in the modern theory of heredity is the distinction between soma and germplasm. You probably know how a strawberry plant sends out runners, or stolons, which grow along the ground indefinitely, sending up at intervals a bud from which a new strawberry plant arises. In time the connecting stolon disintegrates so that each soma becomes separate from the others. The youngest generation is always supplying nutritive material to the further growth of the stolon from which it sprang. This is a picture of the relation of germplasm and soma in man. In every human family is a germplasm that has traversed the ages and has given rise in each generation to one or more somas or persons. Such somas are a sort of biological analysis of the germplasm. Each soma is also a sort of relay station in which the germplasm is maintained and reinvigorated, and from which it is, or may be, started again on its way.

The stream of germplasm is, however, not so simple as the stolon of a strawberry plant. It is a complicated affair, having many tributaries, cross-cuts, and anastomoses. It resembles rather the intricate network of bayous that is characteristic of deltas and other low-lying, irrigated countries. For in all sexually reproducing animals, including man, there is a constant interchange of material from one stream to another. As one sometimes sees a muddy red stream emptying into a clear river and can trace a red streak in the water for miles, so in the mingling of germplasms in sexual reproduction each single character may be traced through generations. Immediately after the union of two germplasms a new soma usually develops, and this combines the characters of both. After the united germplasms have produced the soma they separate again. Consequently the somas arise at the nodal points of an endless

network, and contain characters from two, temporarily united, germplasms—thus in respect to any character the soma is duplex while the germplasm is simplex.

The second fundamental conception in the modern theory of heredity is that of the unit character. These unit characters are the elemental, indivisible hereditary qualities of form or function. We see them in the soma *as generalized qualities*, such as hairiness of the body, pigmentation of the skin, or *as detailed features*, such as the number of digital phalanges, minute facial muscles, and even the different forms of systems of papillary ridges on the hands. Each of these somatic units has its representative or determiner in the germ. It is not always possible by inspection of the soma to say whether an organ or part is composed of only one unit character or of more. But by proper matings the number of elementary determiners may be ascertained in a manner that we may consider later. These unit characters deserve the most careful attention. Incapable of being permanently blended or of intergrading they seem to have arisen suddenly much as we now see them. In poultry, I have seen such unit characters—webbed toes, taillessness, double spurs, extra toes—arise suddenly, complete in the first generation. It is probable that the human characters of taillessness and partial hairlessness and albinism have arisen in the first generation as we see them to-day. Certain rarer human characters such as syndactylism, polydactylism, and complete albinism have almost certainly arisen suddenly, and it is just these characters that are most strikingly inherited.

We are now in a position to formulate our topic more clearly. The study of human heredity is the study of the laws of the development into somatic unit characters of the determiners that are brought together in the union of germplasms.

Now the number of unit characters in man is enormous. A mere catalogue of the more prominent external characters would weary you. Moreover, a complete catalogue would be impossible because of our ignorance. We may, however, recall that characters are both morphological and physiological; normal and teratological. The different organs of the skin—

hairs, pigments, glands, simple sense organs—appear at first to be simple characters. Some of them, however, contain several units. Thus hair has a unit for black pigment and one for red, and perhaps an intensifying factor; it has also a unit for curliness and a factor that stops it from growing beyond a certain length. Doubtless there are other units in the hair alone; and the other dermal organs are doubtless likewise compound. The organs of special sense, like the eye and ear, are special modifications of small portions of the skin; but in these limited areas are concentrated scores of unit characters—units in the membranes limiting the middle ear, in the auditory ossicles, in the basilar and Reissnerian membranes, in the scalæ and in the ducts. The whole body is built up of units, like a mosaic, and each of these is a unit of inheritance. If the determiner of one of these units is lacking in the germplasm or if an additional determiner is present, this peculiarity is inherited. And, whenever the germplasm of one parent differs from that of the other in the presence or absence of even one unit the nature of the offspring will depend on the method of inheritance of that character.

The first and most fundamental law of heredity is this: *Whenever a unit character is absent in both parents, it is absent also in their germplasms and it will be absent in their offspring.* For instance, the white hair of albinos or even flaxen hair results from an absence of black pigment in the hair and indicates an absence of a pigment determiner in the germplasm. Two such parents cannot have dark-haired offspring. Again blue eyes are due to an absence of brown pigment. Two pure blue-eyed parents cannot have brown-eyed children. Straight hair lacks the curling factor. Two perfectly straight-haired parents do not have curly-haired offspring.

The law just stated must be applied with caution. For example, congenital deafness is often indicative of the absence of some unit characters essential to hearing. It might, accordingly, be concluded that two congenital deaf-mutes could have only deaf children; but this is contrary to experience. The fallacy lies in the fact that congenital deafness may be due to

the absence of different factors in the two parents; both may not lack the same character; between them all characters may be represented and, consequently all characters essential to hearing may be present in some or all children. Similarly, two congenitally blind parents may have normal-sighted children. In order that an organ should be defective in all of the offspring it is necessary that there should be the same defect in both parents.

It is probable that the law of absence holds also for psychological characters. I have examined the records of the "Juke" and "Zero" families and it appears, so far as the limited data go, that when ambition, activity and love of work are absent from both parents all of the children are vagabonds and recipients of outdoor relief, although the same mother may have, by an effective criminal, children that stand well in the community. All of these are examples merely of what seems to be a universal rule; namely, that a character absent from both parents cannot reappear in the offspring. In the language of the Mendelian doctrine the parents are *recessive* in respect to the character and two recessive parents produce only recessive offspring. You will not fail to note the importance of this principle in eugenics—the science of proper breeding. Since two parents with the same detrimental defects will have children with these defects it is surely undesirable that they should marry and perpetuate the abnormal condition.

Right here we see the peculiar danger from consanguinity. The chances that two parents defective in any respect, as, for instance, hearing, should lack the same unit character increases the more nearly related they are. In the case of deafness, for instance, which may be due to so many causes, the chances that two congenitally deaf cousins should have the same unit defect is larger than for unrelated couples. Consequently the children of two cousins would more frequently be deaf than those of unrelated parents. This is strikingly the case. Thus, Bell, in his census report on the deaf, finds that "the percentage of congenitally deaf children is nearly three times as great as among those whose parents were not related." Fay finds that

about half (45 per cent.) of consanguinous marriages of deaf parents result in deaf offspring. Thirty per cent. of the children of such marriages are deaf as contrasted with 9 per cent. of the children of non-consanguinous deaf parents. Indeed, it is asserted that parents with more than two congenitally deaf children are almost always close relatives. It seems not improbable, from all the statistics, that whenever both parents are congenitally deaf from the same cause, all of the children will be congenitally deaf.

As with ear troubles, so also with those of the eyes. Relatives are more apt to have the same unit defect. Over one child in every four, it is stated, having *retinitis pigmentosa* is derived from a consanguinous pair. Again, the statistics of the Syracuse Institution, as worked up by Dr. Hrdlicka, show that 16 per cent. of the cases of hereditary feeblemindedness are from consanguinous parents and that there is at least three times as much consanguinity in the parentage of feeble-minded children as in the population at large. Such statistics lend no support to the old view that consanguinity is, in itself, dangerous. As a matter of fact it often seems quite innocuous. This is truest when both parents are from a strain without important defects. The disastrous defects vary in different cases with the nature of the parental weakness. In some experiments with rats incestuous breeding has led to sterility, in others to cripples. In my poultry one inbred strain is characterized by non-resistance to coccidiosis, another is remarkedly infertile. One can imagine cases where, with perfect parents, inbreeding would result in perfect offspring, but with the bodily imperfections found in most families the practice is fraught with danger.

If, in contrast to the foregoing cases, the parent in whom a unit character is lacking be married to one who possesses it, then the unit character will reappear in the offspring. There are, in such cases, two sorts of families. In one sort 100 per cent. of the children possess the character; in the other only 50 per cent. This principle in heredity may be illustrated by eye-color. If in the one parent the iris lacks brown pigment (blue eyes) while in the other it is present then in some

of the families all children will have brown eyes, and in the rest about half the children will have brown eyes and about half blue. Again, if one parent have dark brown or black hair and the other light hair, then it will be found that in some families the children acquire as they grow older dark brown hair, while in other families there is a large proportion of *light brown* and *golden* hair. What is the meaning of this result? When a germ cell with pigment present meets a germ cell with pigment absent, the resulting zygote contains, of course, the pigment forming enzyme and pigment is produced in the hair of the offspring. But, two features of such offspring are to be noted: first, their pigment-forming capacity has been diluted and reduced by the introduction of the unpigmented germ cell (so that the hair of the child will not be so dark as that of the darker parent), and, secondly, the child carries two potentialities of which one is covered over by the other *in the soma*, but both of which are segregated in the germ cells, so that some possess and some lack it. Such a hybrid between the two characters—pigment and no pigment—is, in Mendelian language, a heterozygote; its soma is hybrid but its germ cells are "pure" again—half of them light; half dark. Now, if such a pigmented heterozygote is mated with a consort having no pigment, the pigmentless germ cells of the latter will unite with the two sorts of germ cells of the former in equal numbers. Half of the children will be light; half dark. This gives us the family with an equality of light- and dark-haired children. In the families where all children are dark, the dark parent was not heterozygous. The dark progeny of a light and a dark parent are, however, *all* heterozygotes.

If instead of a heterozygote mated to a light consort we have two heterozygotes mated together then (in large families) the chances are that there will be an equal number of the following four combinations of germ cells. Light x light; light x dark; dark x light, dark x dark. The first combination will produce light children; all of the others, dark. The proportion is 1 to 3. Now, in man (as in other organisms) the various characters of the body are inherited quite independently of each other. Thus

there are straight-haired, blue-eyed individuals, and curly-haired, blue-eyed. As one can predict the proportion of blue eyes in a family, so can one predict that of straight hair in the same family. Two parents, heterozygous in respect to both eye-color and hair form will give to one-quarter of their children light eyes, also to one-quarter straight hair. In the long run only one-fourth of the blue-eyed children will have straight hair and one-quarter of the dark-eyed children will have straight hair; all the others will have twisted hair. Or, in other words, $\frac{1}{4} \times \frac{1}{4}$ (one-sixteenth) will be straight haired and blue eyed; three-sixteenths will be curly haired and blue eyed; three-sixteenths will have straight hair and dark eyes and nine-sixteenths will have curly hair and dark eyes. These proportions are, indeed, actually realized.

It will be seen from the foregoing illustrations that the new knowledge concerning heredity enables us to predict the proportion of children of each kind when the parents and the grandparents are known. The question may now fairly be asked in how far do the principles of heredity here set forth apply to human characters in general? It is clear that if we knew the methods of inheritance of temperamental characters, of various kinds of special ability, even of pathological and teratological characters, it would mark a great advance in eugenics. But, as a matter of fact, very few cases have been worked out. In the case of albinism, which is only an extreme case of absence of pigment, the rule given for blonds holds. In the case of musical ability Hurst finds some evidence that the presence of the talent is dominant over its absence.

In the case of short fingers, involving a reduction in the number of phalanges, the normal condition is recessive. The short fingers seem to be due to an inhibiting factor, an arrest of development, and this dominates over its absence. Similarly syndactylism and the associated phenomenon of split hand is dominant over the normal. Normal children out of an abnormal family of brachydactyls or syndactyls are in no more danger of having abnormal progeny than normals out of any other family. This shows us how important is a knowledge

of the recessive condition in any abnormality, otherwise hardship may be wrought upon recessive individuals who are normal by improperly advising them that there is danger of their transmitting the abnormality.

In the case of bleeding, or hæmophilia, we have an abnormality that is recessive. In this case the abnormality may be carried by a normal person and 100 per cent. of the children of two with abnormal tendencies will be abnormal. Certainly such marriages are bound to be disastrous.

Recently we have been provided by Nettleship [1] with a remarkable pedigree of a family exhibiting night-blindness. The normal condition is recessive, and two normal offspring of abnormal parents produce, when married, no diseased offspring. On the other hand, as Mr. Punnett has lately pointed out [2] in alkaptonuria the diseased condition is probably recessive to the normal, so that two normal (heterozygous) parents may have diseased children.

The general principle that the offspring reveal nothing that is not contributed by the parents seems contradicted by the facts that have long been known under the name of reversion, or harking back to a remote ancestory. The breeders of both plants and animals have long "explained" unexpected results on this ground. Reversion is, however, not different from normal inheritance. When two congenitally deaf parents have hearing children the result is of the class called reversion. But this result is easily accounted for, as already suggested, by the hypothesis that hearing depends on various factors, any one of which being absent deafness ensues. Say, for example, that the factors are A and B. If the father lacks A but has B, while the mother has A but lacks B, then while both parents will be deaf, the offspring (having both A and B) will hear. I may illustrate reversion by a case in poultry. I crossed a white Leghorn cock upon a white silky hen. The offspring were white except for a red wing-bar in the male and a slightly red-

[1] Ophthalmological Society of the United Kingdom Transactions, 1907, xxviii, 269.

[2] Proceedings of the Royal Society of Medicine, March, 1908.

dish breast in the female. I mated the hybrids together, and in the next generation got, among other things, some red and black game-colored birds, almost exactly like the wild jungle-fowl—the reputed ancestor of the domestic fowl. The explanation is simple. Both fowl have the jungle-fowl type of coloring, but in the white Leghorn this is obscured by "graying" while in the white silky a pigment-forming enzyme is apparently lacking. The first generation of hybrids has the pigment-forming enzyme, but its work is obscured by the "graying" factor. In the next generation this is eliminated from certain individuals, which then show the black and red pattern unhindered.

Another complication in heredity is the fact that a heterozygous character is usually different from the pure dominant character. It is sometimes a more or less complete blend. In the second hybrid generation pure dominants reappear, but often the development of a character seems interfered with in such extracted dominants; so that it may stop at an incomplete stage. Thus, dark brown and flaxen hair usually yield some light browns. Such, bred together, produce a brown that is less intense than that of the dark ancestor. It is as though in the extracted dominants the development of the black pigment is stopped prematurely. I imagine such a result will be found to be quite common among people.

A beginning only has been made in the interpretation of the facts of heredity in the light of the new knowledge. The great need is more data; the problem how to get it. There is in this city the greatest commingling of races and characteristics that the world has ever seen. Great is our opportunity therefore to study the inheritance of such diverse characters. Hardly in any other place are there such extensive institutions for criminals, the insane, crippled, blind and deaf, diseased in various ways, poor, and incompetent. Hundreds of superintendents, officers, physicians, and surgeons have the opportunity and sometimes the leisure to ask the critical questions of patients to enter the answers upon blanks and to work up the statistics or turn them over to other students for further study.



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